DOCUMENT-IDENTIFIER: US 20050186109 A1
TITLE: Multilayer analysis element

Detail Description Paragraph:
[0039] For example, when analyzing ammonia (in cases where the tested substance is ammonia or ammonia producing substance), examples of a color ammonia indicator include: leuco dyes, such as leucocyanine dye, nitro-substituted leuco dye, and leucophthalein dye (see U.S. Pat. No. Re. 30267 or JP Patent Publication (Kokoku) No. 58-19062 B (1983); pH indicators, such as bromophenol blue, bromocresol green, bromthymol blue, quinoline blue, and rosolic acid (see Encyclopaedia Chimica, Vol. 10, pp 63-65, published by Kyoritsu Shuppan K. K.); triarylmethane dye precursors; leucobenzylidene dyes (see JP Patent Publication (Kokai) Nos. 55-379 A (1980) and 56-145273 A (1981)); diazonium salt and azo dye couplers; and base bleaching dyes. The content of the color ammonia indicator with respect to the weight of the binder is preferably in the range of about 1 to about 20% by weight.

Detail Description Paragraph:
[0040] The reagent that reacts with an ammonia producing substance as a tested substance to produce ammonia is preferably an enzyme or a reagent that contains an enzyme, and the enzyme suitable for analysis may be selected appropriately depending on the type of the ammonia producing substance as the tested substance. When an enzyme is used as the regent, the combination of the ammonia producing substance and the reagent is determined by the specificity of the enzyme. Examples of the combination of the ammonia producing substance and the enzyme as the reagent include: urea/urease; creatinine/creatinine deiminase; amino acid/amino-acid dehydrogenase; amino acid/amino-acid oxidase; amino acid/ammonia lyase; amine/amine oxidase; diamine/amine oxidase; glucose and pho sphoamidate/phosphoamidate-hexo se pho sphotran sferase; ADP/carbamate kinase and carbamoyl phosphate; acid amide/amide hydrolase; nucleobase/nucleobase deaminase; nucleoside/nucleoside deaminase; nucleotide/nucleotide deaminase; guanine/guanase. An alkaline buffer that can be used in the reagent layer during the analysis of ammonia may be a buffer with a pH of 7.0 to 12.0, and preferably 7.5 to 11.5.

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DOCUMENT-IDENTIFIER: US 5286624 A
TITLE: Integral multilayer analytical element for determination of ammonia or ammonia-producing substance

Brief Summary Text (5):
As the process for producing ammonia from an ammonia-producing substance, a common method is of producing ammonia by the action of an enzyme. For example, creatinine in a biological body fluid is determined by utilizing creatinine deiminase (EC 3.5.4.21) which hydrolzes specifically creatinine in the body fluid to ammonia and N-methylhydantion. Urea nitrogen (BUN) in a biological body fluid is determined by utilizing urease which hydrolyzes urea to ammonia and carbon dioxide. In the above methods, since the ammonia-producing substance, which is an analyte, is a substrate of an enzyme, the substance is called also ammonia-producing substrate. Analytical methods of the ammonia-producing substance are described in various references, such as "Analytical Chemistry", 46, 246 (1974), "Climica Clinica Acta", 18, 409 (1967), "Rinsho Kagaku Bunseki III Gan-Chisso Seibun (Clinical Chemical Analysis III Nitrogen-Containing Components) 2nd Edition", Tokyo Kagaku Dojin, Tokyo, 13-14, 67-87 (1979) and "Rinsho Kensa (Journal of Medical Technology)" 5(6), 387-391 (1961).

Detailed Description Text (7):
The coloring ammonia indicator usable for the integral multilayer analytical element of the invention includes leuco dyes, such as, leuco cyanine dye, nitro-substituted leuco dye, and leuco phthalein dye,,disclosed in U.S. Pat. No. Reissue No. 30 267 or Japanese Patent KOKOKU No. 58-19062, pH indicators, such as, Bromophenol Blue, Bromocresol Green, Bromthymol Blue, Quinoline Blue and rosolic acid disclosed in "Kagaku Dai Jiten, Encyclopaedia Chimica", vol. 10, pp 63-65, Kyoritsu Shuppan, Tokyo, 1962, triarylmethane dye precursors, leuco benzylidene dyes disclosed in Japanese Patent KOKAI No. 55-379 or 56-145273), diazonium salts and azo dye couplers, and alkali-bleachable dyes.

Detailed Description Text (25):
The reagent reacting with an ammonia-producing substance to produce ammonia is preferably an enzyme or a reagent containing an enzyme, and the enzyme suitable for the analysis can be selected according to the kind of the ammonia-producing substance which is the analyte. In the case of using an enzyme as the above reagent, the combination of ammonia-producing substance and reagent is decided by the specificity of the enzyme. Examples of ammonia-producing substance/reagent are urea/urease, creatinine/creatinine deiminase, amino acid/amino acid dehydrogenase, amino acid/amino acid oxidase, amino acid/ammonia lyase, amine/amine oxidase, diamine/amine oxidase, glucose and phosphoamidate/phosphoamidate hexose phosphotransferase, ADP/carbamate kinase and carbamoylphosphate, acid amide/amide hydrolase, nucleobase/nucleobase deaminase, nucleoside/nucleoside deaminase, nucleotide/nucleotide deaminase, guanine/guanase, etc.

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US005286624A

United States Patent [19]

Terashima et al.

[11] **Patent Number: 5,286,624**

[45] **Date of Patent: Feb. 15, 1994**

[54] **INTEGRAL MULTILAYER ANALYTICAL ELEMENT FOR DETERMINATION OF AMMONIA OR AMMONIA-PRODUCING SUBSTANCE**

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[73] Assignee: **Fuji Photo Film Co., Ltd.**, Kanagawa, Japan

[21] Appl. No.: **779,363**

[22] Filed: **Oct. 17, 1991**

[30] **Foreign Application Priority Data**

Oct. 19, 1990 [JP] Japan 2-281087

[51] **Int. Cl.**[5] **C12Q 1/58**; C12Q 1/50; G01N 21/77

[52] **U.S. Cl.** **435/12**; 435/17; 435/805; 422/56; 422/57; 436/169; 436/170; 526/332

[58] **Field of Search** 435/12, 17, 805; 422/56, 57; 436/169, 170; 526/332

[56] **References Cited**

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Primary Examiner—Michael G. Wityshyn
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[57] **ABSTRACT**

An integral multilayer analytical element for the determination of ammonia or an ammonia-producing substance comprising a light-transmissive liquid-impermeable support, an indicator layer containing an indicator which produces a detectable change by gaseous ammonia, a liquid permeation barrier layer, a reagent layer containing an alkaline buffer and optionally a reagent capable of reacting with a substance to produce ammonia and a spreading layer laminated in this order, which is improved by that the indicator layer contains a polyvinyl alkyl ether, and/or which is improved by that the surface of said support facing toward the indicator layer is undercoated with a polyvinyl alkyl ether, a hydroxyalkyl cellulose, an alkyl cellulose, polystyrene, a polyalkyl methacrylate, polyviriylidene chloride, polyvinyl alcohol or polyvinyl pyrrolidone, substantially not containing ammonia and ammonium ion. By using the above analytical element, ammonia or an ammonia-producing substance can be analyzed at a high coloring optical density and a high accuracy. The measuring accuracy is further improved by lowering the background optical density.

14 Claims, No Drawings

INTEGRAL MULTILAYER ANALYTICAL ELEMENT FOR DETERMINATION OF AMMONIA OR AMMONIA-PRODUCING SUBSTANCE

BACKGROUND OF THE INVENTION

This invention relates to an integral multilayer analytical element for the determination of ammonia or an ammonia-producing substance in a liquid sample. More particularly, this invention relates to an integral multilayer analytical element suitable for the determination of ammonia or an ammonia-producing substance such as creatinine or urea contained in a biological body fluid such as blood, urine or lymph.

The quantitative analysis of ammonia, creatinine, urea or the like is very important for the diagnosis of various diseases such as nephropathy, the inspection for the medical treatment course of the disease, and the inspection of renal functions.

A representative analytical method for an ammonia-producing substance comprises a process for producing ammonia from the ammonia-producing substance and a process for determining the produced ammonia. The analytical method of utilizing the conversion to ammonia has been widely utilized in the so-called wet analysis or the solution method. Recently, in the dry analysis using a dry analytical device represented by integral multilayer analytical elements, the analytical method of utilizing the conversion to ammonia has been applied or proposed.

As the process for producing ammonia from an ammonia-producing substance, a common method is of producing ammonia by the action of an enzyme. For example, creatinine in a biological body fluid is determined by utilizing creatinine deiminase (EC 3.5.4.21) which hydrolzes specifically creatinine in the body fluid to ammonia and N-methylhydantion. Urea nitrogen (BUN) in a biological body fluid is determined by utilizing urease which hydrolyzes urea to ammonia and carbon dioxide. In the above methods, since the ammonia-producing substance, which is an analyte, is a substrate of an enzyme, the substance is called also ammonia-producing substrate. Analytical methods of the ammonia-producing substance are described in various references, such as "Analytical Chemistry", 46, 246 (1974), "Climica Clinica Acta", 18, 409 (1967), "Rinsho Kagaku Bunseki III Gan-Chisso Seibun (Clinical Chemical Analysis III Nitrogen-Containing Components) 2nd Edition", Tokyo Kagaku Dojin, Tokyo, 13–14, 67–87 (1979) and "Rinsho Kensa (Journal of Medical Technology)" 5(6), 387–391 (1961).

Integral analytical elements usable for the analysis of ammonia or an ammonia-producing substrate are the integral multilayer analytical element described in Japanese Patent KOKOKU No. 58(1983)-19062 (U.S. Pat. No. Reissue No. 30,267), the integral multilayer analytical elements for the analysis of ammonia or an ammonia-producing substrate disclosed in U.S. Pat. No. 4,548,906 (Japanese Patent KOKAI No. 58(1983)-77661) and Japanese Patent KOKAI No. 58(1983)-77660 and the like. The fundamental construction of the above analytical elements is composed of a light-transmissive, liquid-impermeable support, an ammonia indicator layer containing an indicator which produces a detectable change by contacting ammonia, a liquid permeation barrier layer which is permeable to gaseous ammonia and substantially impermeable to liquid, a reaction layer containing a reagent which reacts with an ammonia-producing substrate to produce ammonia and a porous spreading layer laminated in this order. The integral multilayer analytical element disclosed in EP 0 204 334 A has a trapping function of the ammonia contained in a body fluid (endogeneous ammonia) in the analytical element itself, and the analytical element can remove the influence of the endogeneous ammonia. This analytical element is provided with an endogeneous ammonia-trapping layer which conducts an ammonia-trapping reaction above the layer generating ammonia through an ammonia-producing reaction in contact therewith. The integral multilayer analytical element disclosed in EP 0 287 112 A is provided with a layer having a diffusion-preventing ability which does not perform trapping of ammonia and ammonia-producing reaction between the endogeneous ammonia-trapping layer and the ammonia-producing reaction reagent layer.

Heretofore, a copolymer latex of polyvinyl acetate-acrylate ester was used as the binder of the indicator layer. However, the analytical element using the above copolymer latex is insufficient in sensitivity, and the accuracy (CV=variation coefficient) is inferior. Since the coating solution is latex, it is inferior in the liquid stability because of the occurrence of precipitation. Moreover, since a pH variation of the latex solution occurs, this analytical element is unsuitable for the system using a pH indiator.

SUMMARY OF THE INVENTION

An object of the invention is to provide an integral multilayer analytical element for the determination of ammonia or an ammonia-producing substance of which the developed color density is higher and the measuring accuracy is higher.

Another object of the invention is to provide an integral multilayer analytical element for the determination of ammonia or an ammonia-producing substance of which the background color density is low and the measuring accuracy is thereby further improved.

The present invention has achieved the above objects, and provided an integral multilayer analytical element for the determination of ammonia or an ammonia-producing substance comprising a light-transmissive liquid-impermeable support, an indicator layer containing an indicator which produces a detectable change by gaseous ammonia, a liquid permeation barrier layer, a reagent layer containing an alkaline buffer and optionally a reagent capable of reacting with a substrate to produce ammonia and a spreading layer laminated in this order, which is improved in that the indicator layer contains a polyvinyl alkyl ether.

The present invention also provides an integral multilayer analytical element for the determination of ammonia or an ammonia-producing substance comprising a light-transmissive liquid-impermeable support, an indicator layer containing an indicator which produces a detectable change by gaseous ammonia, a liquid permeation barrier layer, a reagent layer containing an alkaline buffer and optionally a reagent capable of reacting with a substrate to produce ammonia and a spreading layer laminated in this order, which is improved in that the surface of said support on the indicator layer side is undercoated with a polyvinyl alkyl ether, a hydroxyalkyl cellulose, an alkyl cellulose, polystyrene, a polyalkyl methacrylate, polyvinylidene chloride, polyvinyl

alcohol or polyvinyl pyrrolidone, substantially not containing ammonia and ammonium ion.

DETAILED DESCRIPTION OF THE INVENTION

The light-transmissive liquid-impermeable suport is composed of polyethylene terephthalate, polycarbonate of bisphenol A, polystyrene, cellulose ester (for example, cellulose diacetate, cellulose triacetate or cellulose acetate propionate, etc.), and the thickness is in the range of about 50 μm to about 1 mm, preferably about 80 μm to about 300 μm.

An undercoat layer containig a particular polymer can be provided on the surface of the support in order to render the adhesion of the indicator layer to the support tight. The analytical element of the invention is characterized by using a polyvinyl alkyl ether, a hydroxyalkyl cellulose, an alkyl cellulose, polystyrene, a polyalkyl methacrylate, polyvinylidene chloride, polyvinyl alcohol or polyvinyl pyrrolidone, substantially not containing ammonia and ammonium ion, instead of gelatin as the undercoat layer. The polyvinyl alkyl ether is polyvinyl methyl ether, polyvinyl ethyl ether, polyvinyl isobutyl ether or the like, and the hydroxyalkyl cellulose is hydroxymethyl cellulose, hydroxyethyl cellulose. The alkyl cellulose is methyl cellulose, ethyl cellulose or the like. Among them, the polyvinyl alkyl ethers are the most preferred.

The undercoat layer is composed of the above polymer and an anchoring agent which is optionally added. As the anchoring agent, an organic solvent which allows swelling or dissolves the support can be used. In the case where the support is polyethylene terephthalate, suitable anchoring agents are phenol, phenol deriatives, such as, o-chlorophenol, p-chlorophenol and cresol, 2-nitropropanol, acetonylacetone, acetophenone, etc. The undercoat layer can be provided by dissolving the polymer and the anchoring agent in a solvent, such as, methanol, ethanol, methyl cellosolve (B-hydroxyethylmethyl ether) or acetone in a polymer concentration of about 0.1% to about 2%, preferably about 0.1% to about 1%, applying the above undercoating solution by a known method followed by drying. The coating amount of the polymer is usually about 5 mg/m^2 to about 500 mg/m^2, preferably about 10 mg/m^2 to about 300 mg/m^2. The ratio of the polymer to the anchoring agent is about 1:10 to about 1:100 by weight.

Prior to applying the undercoating solution, the surface of the support may be provided with a physical activation treatment, such as, glow discharge or ultraviolet irradiation, or a chemical activation treatment.

The indicator layer (ammonia indicator layer) containing an indicator which produces a detectable change by gaseous ammonia is provided on the support directly or through the undercoat layer. The indicator layer contains at least one kind of coloring ammonia indicator. The coloring ammonia indicator is a compound which produces a detectable change, such as, coloring or color change due to the change of absorption wave length, by gaseous ammonia.

The coloring ammonia indicator usable for the integral multilayer analytical element of the invention includes leuco dyes, such as, leuco cyanine dye, nitro-substituted leuco dye, and leuco phthalein dye,,disclosed in U.S. Pat. No. Reissue No. 30 267 or Japanese Patent KOKOKU No. 58-19062, pH indicators, such as, Bromophenol Blue, Bromocresol Green, Bromthymol Blue, Quinoline Blue and rosolic acid disclosed in "Kagaku Dai Jiten, Encyclopaedia Chimica", vol. 10, pp 63-65, Kyoritsu Shuppan, Tokyo, 1962, triarylmethane dye precursors, leuco benzylidene dyes disclosed in Japanese Patent KOKAI No. 55-379 or 56-145273), diazonium salts and azo dye couplers, and alkali-bleachable dyes.

The indicator layer is formed by preparing a coating solution by mixing at least one kind of the coloring ammonia indicator with an organic solvent-soluble binder polymer or a water-soluble binder polymer, and applying the coating solution onto the support followed by drying. Heretofore, the binder polymer used was a cellulose ester, such as, cellulose monoacetate, cellulose diacetate, cellulose triacetate, cellulose acetate butyrate or cellulose propionate, an alkyl cellulose, such as, methyl cellulose, ethyl cellulose or propyl cellulose a synthetic vinyl polymer, such as, polymethyl methacrylate, polyacrylate, polystyrene, polyacrylonitrile, polyvinyl chloride, polyvinyl butyral, chlorinated polyvinyl acetate, polyacrylamide, polyvinyl pyrrolidone or polyvinyl alchol, or a copolymer thereof. The analytical element of the invention is characterized by using a polyvinyl alkyl ether as the binder polymer. Examples of the polyvinyl alkyl ether are polyvinyl methyl ether, polyvinyl ethyl ether, polyvinyl isobutyl ether and the like.

The blending amount of the coloring ammonia indicator is preferably 1 to 20 wt. % of the binder polymer. In order to prevent coloring or discoloration of the coloring ammonia indicator during manufacturing or storing, the pH value at the indicator layer can be controlled to the coloring pH range of the coloring ammonia indicator by adding an organic acid or inorganic acid, such as, ethanesulfonic acid, aspartic acid, azelaic acid, glutaric acid, succinic acid, glutaconic acid, tartaric acid, pimelic acid, malonic acid, malic acid, 3,3-dimethylglutaric acid, citric acid, p-toluenesulfonic acid, perchloric acid or chloric acid or an alkali, such as, sodium hydroxide, potassium hydroxide, disodium carbonate or sodium hydrogen carbonate to the indicator layer.

The coating solution forming the indicator layer can be prepared by adding reagents, such as, the coloring pH to an organic solvent, such as, acetone, 2-methyoxyethanol, methyl ethyl ketone, dichloromethane, dichloroethane, methanol or ethanol or water in a solids concentration of usually about 1 to 30 wt. %, preferably about 3 to 20 wt. The indicator layer can be formed by applying the coating solution onto the support so that the dry thickness becomes usually about 1 to 30 μm, preferably about 2 to 20 μm, followed by drying.

The liquid permeation barrier layer (barrier layer) which can pass geseous ammonia is provided on the indicator layer. The barrier layer means that the layer composed of a material which substantially does not pass the liquid components and interfering substances dissolved in the liquid components, such as, alkaline components, of the coating solution and a sample liquid but passes gaseous ammonia through manufacturing the multilayer analytical element, actually at the time of providing the reaction layer described later on the barrier layer by applying and/or through analytical operations.

The barrier layer can be divided into two embodiments in the structural sense. One embodiment is an air barrier layer which is constructed by a porous material having continuous pores wherein an air layer substantially acts as the barrier layer, and the other embodi-

ment is a polymer barrier layer which is a homogeneous nonporous thin layer constructed by a hydrophobic or less hydrophilic polymer.

Examples of the porous material having continuous pores composing the air barrier layer are: a membrane filter, a porous material formed by a fibrous material entangled with, adhered to or bonded to each other, such as, paper, filter paper, felt or nonwoven fabric, a porous material composed of a woven fabric, a knitted fabric or a fine net material.

The membrane filters usable as the air barrier layer are those produced by using a cellulose acetate, such as, cellulose diacetate or cellulose triacetate, cellulose nitrate, regenerated cellulose, polyamide (nylon), polycarbonate of bisphenol A, polyethylene, polyproplyene, a fluorine-containing polymer, such as, polytetrafluoroethylene, or the like. In the case of using the membrane filter for the integral multilayer analytical element of the invention, a suitable thickness is in the range of usually about 30 to 300 μm, preferably 70 to 200 μm. The porosity (void content) of the membrane filter is usually about 25 to 90%, preferably about 60 to 90%. The mean pore size of the membrane filter is in the range of usually about 0.01 to 20 μm, preferably about 0.1 to 10 μm. Membrane filters having the above properties can be prepared according to the method described in U.S. Pat. No. 1,421,341 or U.S. Pat. No. 3,992,158. Besides, various membrane filters are supplied by many manufactures, and the membrane filter can be selected therefrom.

As the porous material formed by a fibrous material entangled with, adhered to or bonded to each other usable as the air barrier layer, there are the porous materials having continuous pores composed of a fibrous material or an aggregate thereof which is physically entangled or physically and/or chemically adhered or bonded, disclosed in Japanese Patent KOKAI No. 58-77660.

Examples of the fibrous material composing the above porous material are natural fibrous materials, such as, cellulose fiber, cotton fiber, hemp fiber, silk fiber and wool fiber, regenerated fibers and semisynthetic fibers, such as, rayon fiber, vinylon fiber and cellulose acetate fiber, synthetic materials, such as, glass fiber, polyethylene fiber, polyethylene terephthalate fiber, polyacrylonitrile fiber and polyvinyl chloride fiber, and fiber materials composed of a mixture of them. As the examples of the porous material produced by using the above fibrous material, there are papers, such as Japanese papers including rice paper(traditional Japanese writing paper), mino paper and shoji paper, filter paper, parchment paper and artificial parchment paper, felt, nonwoven fabric, and the like, made of the fibrous material.

The void content of the porous material formed by a fibrous material entangled with, adhered to or bonded to each other is in the range of usually about 20 to 90%, preferably about 50 to 85%. The mean pore size of the above porous material is in the range of usually about 0.01 to 20 μm, preferably about 0.1 to 10 μm. The thickness of the above porous material is in the range of usually about 50 to 500 μm, preferably about 70 to 300 μm.

As the examples of the woven fabric usable as the air barrier layer, there are various woven fabrics made of a natural fiber, such as, cotton broad cloth, various woven fabrics made of a semisynthetic fiber, such as, broad cloths made of regenerated cellulose fiber, e.g. viscose rayon, cuprammonium rayon or Fortisan, various woven fabrics made of a synthetic fiber, such as, broad cloths made of polyamide (nylon), polyethylene teraphthalate or polyacrylonitrile, and blended yarn woven fabrics of a natural fiber with a semisynthetic fiber or synthetic fiber, such as, broad cloth made of the blended yarn of silk fiber and polyethylene terephthalate fiber. As the examples of the knitted fabric usable as the air barrier layer, there are various knitted fabrics made of the same fiber or twist yarn thereof as the fiber usable for the production of the aforementioned woven fabrics. As examples of the fine net material usable as the air barrier layer, there are various fine nets and fine meshes made of a synthetic fiber or yarn, such as polyamide (nylon), polyethylene terephthalate, polyacrylonitrile, polyethylene, polypropylene or polyvinyl chloride. The thickness of the above woven fabric, knitted fabric and fine net material is usually in the range of about 30 to 300 μm. The void content of the woven fabric, knitted fabric and fine net material is usually about 20 to 60%, preferably about 40 to 60.

In the above air barrier layer composed of the porous material having continuous pores, there is a possibility that liquid components, particularly those containing interfering materials, such as, alkaline materials, pass the inner space of the barrier layer by capillary phenomena. Therefore, it is preferred that the air barrier layer has a hydrophobic property or water repellency to the degree not to generate capillary flow by capillary phenomena. When the hydrophobic property or water repellency of the porous material having continous pores is weak, it is preferable to conduct a treatment to render it hydrophobic or water-repellent. The treatment to render the porous material hydrophobic or water-repellent can be conducted by using a common agent used for hydrophobic treatment or a common water repellent, represented by silicone resin, silicone oil, fluorine contained resin and fluorine contained oil as it is or optionally diluted with a solvent, such as, hexane, cyclohexane or petroleum ether in a solid content of about 0.1 to 5 wt. %, and applying it onto at least one surface in the neighborhood of the porous material having continuous pores by immersing, coating or spraying.

The air barrier is formed by adhering the porous material having continuous pores to the aforementioned organic solvent-soluble binder polymer or water-soluble binder polymer or water-soluble binder polymer composing the matrix of the indicator layer. The above adhesion of the porous material can be conducted by adhering the porous material to the indicator layer when it is in a wet state, and then drying. The indicator layer in a wet state means that the binder polymer composing the matrix of the indicator layer is in a wet state, dispersed state or solution state by the solvent used for dissolving the binder polymer which still remains on wetting the dried membrane with a soluble solvent, such as an organic solvent or water. When the binder polymer of the indicator layer is adhesive, such as, polyvinyl acetate, the porous material having continuous pores can be adhered to the indicator layer by pressing without wetting the indicator layer.

The polymer barrier layer is preferably composed of a hydrophobic or less hydrophilic polymer. Examples or the hydrophobic or less hydrophilic polymer are cellulose acetate propionate, cellulose acetate butyrate, polycarbonate of bisphenol A, polyethylene, polypropylene, ethylene-vinyl acetate copolymer, polyure-

thane, polystyrene, polyvinyl chloride, vinyl chloride-vinyl acetate copolymer, polyamide (nylon), polymethyl methacrylate and polyvinyl butyral. These polymers may be blended with each other.

The thickness of the polymer barrier layer is usually in the range of about 0.1 to about 6 μm, preferably about of 0.2 to about 3 μm. The polymer barrier layer can be provided by applying a polymer organic solvent solution and then drying, according to the method disclosed in Japanese Patent KOKOKU No. 58-19062 or Japanese Patent KOKAI No. 60-21452.

Preferable barriers are air barrier layer of the membrane filter composed of a vinyl polymer, such as, polyethylene or polypropylene or a fluorine-containing vinyl polymer, such as, polytetrafluoroethylene, treated with or without a water repellent, in view of short analytical operation time, high sensitivity and uniform coloring or discoloration of the indicator layer.

The reagent layer is provided on the barrier layer directly or through an adhesive intermediate layer described later. The reagent layer usually contains, in the case of the analytical element for the determination of an ammonia-producing substance, a reagent reacting with the ammonia-producing substance to produce ammonia which is generally an enzyme or a reagent containing an enzyme, an alkaline buffer for releasing the ammonia produced through the reaction as gaseous ammonia efficiently and a hydrophilic polymer binder having a film-forming ability. In the case of the analytical element for the determination of ammonia, the above reagent for producing ammonia is not necessary.

The reagent reacting with an ammonia-producing substance to produce ammonia is preferably an enzyme or a reagent containing an enzyme, and the enzyme suitable for the analysis can be selected according to the kind of the ammonia-producing substance which is the analyte. In the case of using an enzyme as the above reagent, the combination of ammonia-producing substance and reagent is decided by the specificity of the enzyme. Examples of ammonia-producing substance/reagent are urea/urease, creatinine/creatinine deiminase, amino acid/amino acid dehydrogenase, amino acid/amino acid oxidase, amino acid/ammonia lyase, amine/amine oxidase, diamine/amine oxidase, glucose and phosphoamidate/phosphoamidate hexose phosphotransferase, ADP/carbamate kinase and carbamoylphosphate, acid amide/amide hydrolase, nucleobase/nucleobase deaminase, nucleoside/nucleoside deaminase, nucleotide/nucleotide deaminase, guanine/guanase, etc.

The alkaline buffer usable for the reagent layer is usually in the range of pH 7.0 to 12.0, preferably 7.5 to 11.5. Examples of the buffer are ethylenediaminetetraacetic acid (EDTA), tris(hydroxymethyl)aminomethane (Tris), phosphate buffer, N,N-bis(2-hydroxyethyl)glycine (Bicine), N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfuric acid (Taps), N-2-hydroxyethylpiperazine-N'-2-hydroxypropane-3-sulfonic acid (Heppso), N-2-hydroxyethylpiperazine-N'3-propane sulfonic acid (Epps), N,N-bis(2-hydroxyethyl)-2-aminoethane sulfonic acid, 3-[N-bis(hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (Dipso), N-hydrosyethylpiperazine-N'-ethanesulfonic acid (Hepes), piperazine-N,N'-bis(2-hydroxypropanesulfonic acid) dehydrate (Popso), 3-[N-tris(hydroxymethyl)methylamino]-2-hydroxypropanesulfonic acid (Tapso), N-tris(hydroxymethyl) methylaminoethanesulfonic acid (Tes), N-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine (Tricine) and the like, their alkali metal salts, such as, lithium salts sodium salts and potassium salts, their alkaline earth metal salts, alkali metal salts of tetraboric acid, such as, sodium tetraborate, disodium carbonate-sodium hydrogen carbonate, and the like. The details of the above buffers are described in Biochemistry, 5, 467–477 (1966). Analytical Biochemistry, 104, 300–310 (1980), The Chemical Society of Japan, "Kagaku-Benran, Kiso-Hen (Chemical Handbook, Fundamental Volume)", 1312–1320, Maruzen, Tokyo (1966), etc.

The hydrophilic polymer binder having a film-forming ability usable for the reagent layer is preferably a binder which substantially does not contain ammonia, which substantially does not generate ammonia at a pH of not less than about 9.0 (pH value≧9.0) and of which the binder ability substantially does not vary at a pH of not less than about 9.0.

The about pH 9.0 means the pH where NH_4^+ can be sufficiently converted to NH_3 ($NH_4^+ \rightarrow NH_3$; pKa=9.2). That the binder ability substantially does not vary at a pH of not less than about 9.0 means that, even when a solution containing the binder is left for a long period in the circumstance of a pH of not less than about 9.0, the viscosity scarsely varies, e.g. at pH 10.0 at 45° C. after 24 hours, the viscosity variation is not more than 1%.

The hydrophilic polymer binder can be selected from the polymers having hydrophilic property among the aforementioned water-soluble binder polymers usable for the indicator layer. Other hydrophilic polymers usable for the reagent layer are gelatin, gelatin derivatives, agarose, pullulan, pullulan derivatives, hydroxyalkyl celluloses, alkyl celluloses, polyvinyl alcohol, polyacrylamide, etc. Among these polymers, hydroxyalkyl celluloses are, in general, preferred.

The reagent layer can contain optionally a wetting agent, a binder crosslinking agent (curing agent), a stabilizer, a heavy metal ion trapping agent (chelating agent), and the like, in addition to the reagent reacting with an ammonia-producing substance to produce ammonia, the alkaline buffer and the hydrophilic polymer binder having a film-forming ability. The heavy metal ion trapping agent is used for masking the heavy metal ion which inhibits enzyme activity. Examples of the heavy metal ion trapping agent are complexanes, such as, EDTA.2Na, EDTA.4Na, nitrilotriacetic acid (NTA) and diethylenetriaminepentaacetic acid.

The reagent layer can be formed by preparing a coating solution by mixing the reagent reacting with an ammonia-producing substance to produce ammonia, the alkaline buffer and the above optional reagents with the hydrophilic polymer binder having a film-forming ability, such as a hydroxyalkyl cellulose, polyvinyl alcohol or agarose, and applying it onto the barrier layer or an adhesive intermediate layer followed by drying.

The amount of the reagent reacting with an ammonia-producing substance to produce ammonia contained in the reagent layer is usually about 0.1 to 50 wt. %, preferably about 0.2 to 20 wt. % of the weight of the polymer binder. A suitable amount of the alkaline buffer is in the range of 1.0 to 50 wt. % of the weight of the polymer binder. In the case of using the heavy metal ion trapping agent, a suitable amount is about 0.5 to 20 wt. % of the weight of the polymer binder. The dry thickness of the reagent layer is usually in the range of 1 to 30 μm, preferably 2 to 20 μm.

The adhesive intermediate layer which can be provided between the barrier layer and the reagent layer is composed of a polymer composition which exhibits adhesive property in the atmosphere at a humidity of 10 to 85% at a usual environmental temperature (about 0° to 400° C.). The above adhesive intermediate layer can be provided acording to the materials and method described in Japnese Patent KOKAI No. 60-21452. The polymer composition forming the adhesive intermediate layer is a mixture composed of one or more kinds of a known polymer having a glass transition point (Tg) of not higher than 0° C. and an optional tackfier and an optional surfactant and the like. The thickness of the adhesive intermediate layer is usually in the range of about 0.1 to 6 μm, preferably 1 to 4 μm.

As the example of the polymer usable for the adhesive intermediate layer, there are vinyl acetate-butylacrylate copolymer, poly(ethylacrylate), styrene-butylacrylate-acrylic acid-N-(hydroxymethyl)acrylamide quaternary copolymer, butylacrylate-(ethylacetacetate)methacrylate-2-acrylamide-2-methylpropanesulfonic acid ternary copolymer, and the like.

When the barrier layer is the polymer barrier layer which is a homogeneous nonporous thin layer of a hydrophobic or less hydrophilic polymer, it is preferable to provide the ahesive intermediate layer.

On the reagent layer, it is preferable to provide an ammonia diffusion-preventing layer which has an ability to substantially prevent (or hinder) the ammonia generated in the reagent layer from diffusing into the ammonia-trapping layer described later and which substantially does not conduct trapping of ammonia and ammonia-producing reaction.

The ammonia diffusion-preventing layer may be replaced by another layer having a different function which substantially does not conduct trapping of ammonia and ammonia-producing reaction. The layer having a different function includes a hardened (or cross-linked) hydrophilic polymer layer, a light blocking layer and an adhesive layer.

The polymer binder usable for the ammonia diffusion-preventing layer can be selected arbitrarily from those having a film-forming ability, the film being preferably substantially nonporous and water-permeable and capable of being recoated onto the reagent layer directly or through a suitable intermediate layer. Among the aforementioned polymer binders usable for the indicator layer or the reagent layer, those capable of substantially passing water can be used for this layer. Preferred polymer binders are the hydrophilic polymers used for the reagent layer, and the hydrophilic polymers swelling with water or being water-soluble are particularly preferred. Preferred hydrophilic polymers are hydroxyalkyl celluloses, agarose, polyvinyl alcohol and the like.

The ammonia diffusion-preventing layer may be formed of a polymer binder alone, but it is preferred to control the pH value between about 7.0 to 12.0 by adding a suitable buffer, in order to improve the efficiency for hindering ammonia diffusion. Particularly preferable pH value is in the range of about 8.0 to 11.0.

The buffering ability for maintaining the pH value of the ammonia diffusion-preventing layer in the above range may be any one having a buffering ability in the above pH range. As examples of the buffer usable for hindering ammonia diffusion, there are alkaline buffers and alkaline agents similar to those usable for the aforementioned reagent layer.

The ammonia diffusion-preventing layer is preferably thick in view of hindering ammonia diffusion, whereas it is preferably thin in view of water permeation. Therefore, a suitable thickness is selected so as to satisfy both functions.

The ammonia diffusion-preventing layer is, in the case of a substantially nonporous layer containing a hydrophilic polymer binder, in a thickness of about 2 to 50 μm, preferably about 4 to 30 μm, and the coating amount of the polymer binder is about 1.5 to 40 g, preferably about 3.0 to 25 g per 1 m^2 of the analytical element.

In the case of the analytical element for the determination of an ammonia-producing substance, it is preferable to provide an endogeneous ammonia-trapping layer containing a reagent acting on the ammonia already present in an aqueous liquid sample (endogeneous ammonia) to convert it to a state where it is substantially impossible for the ammonia to reach the aforementioned reagent layer, on the ammonia diffusion-preventing layer directly or through a light blocking layer or another intermediate layer. The endogeneous ammonia-trapping layer has a function of trapping the coexisting endogeneous ammonia, prior to the occurrence of the reaction producing ammonia by reaction of the analyte of an ammonia-producing substance, such as, creatinine or urea nitrogen, to the reagent layer.

To trap the endogeneous ammonia means that the reagent system contained in the endogeneous ammonia-trapping layer is bound to render the endogeneous ammonia in a substantially unreleasable state through the analytical operations, or that the reagent system contained in the endogeneous ammonia-trapping layer reacts with the endogeneous ammonia to convert it to another chemical substance, actually a chemical substance different from ammonium salt, ammonium ion and gaseous ammonia, to fix the endogeneous ammonia to the endogeneous ammonia-trapping layer, and thereby, substantially inhibits the endogeneous ammonia to reach the reagent layer. The endogeneous ammonia-trapping layer preferably contains the latter reagent system having a function to react with the endogeneous ammonia to fix it. In this specification, the reagent system reacting with an endogeneous ammonia to convert it to another chemical substance is called an endogeneous ammonia-trapping reagent.

As the endogeneous ammonia-trapping reagent, the reagent compositions containing an enzyme having a catalytic ability acting on ammonia as a substrate to convert it to another substance are preferred. Examples of the endogeneous ammonia-trapping reagent are reagent compositions containing NADH (nicotinamide adenine dinucleotide in reduced form) and/or NADPH (nicotinamide adenine dinucleotide phosphate in reduced form), glutamate dehydrogenase (EC 1.4.1.3;GlDH) and α-ketoglutaric acid or its sodium salt (α-KG). Reagent compositions containing aspartase (EC 4.3.1.1) and fumaric acid or a fumarate salt may also be usable. In the integral multilayer analytical element of the invention, it is preferable to use a reagent composition containing NADH, GlDH and α-KG as the endogeneous ammonia-trapping reagent. In the case of using a reagent composition containing GlDM or a reagent composition containing aspartase, it is preferable to use a suitable buffer so as to maintain the pH value of the endogeneous ammonia-trapping layer to usually not higher than 10.0, preferably in the range of 7.0 to 9.5.

As the buffer for maintaining the above pH value usable for the endogeneous ammonia-trapping reagent, there are the buffers described in The Chemical Society of Japan, "Kagaku-Benran, Kiso-Hen (Chemical Handbook, Fundamental Volume)", 1312–1320, Maruzen, Tokyo (1966), the buffers described in Norman E. Good, et al, Biochemistry, 5 (2), 467–477 (1966), Hydrogen Ion Buffers for Biological Research, the buffers described in R. M. G. Davson et al, Date for Biochemical Research, Second Edition, 476–508, Oxford at the Clarendon Press (1919), the buffers described in Analytical Biochemistry, 104, 300–310 (1980), etc. Besides, organic acids and their alkali metal or alkaline earth metal salt usable for the integral multilayer analytical element described in Japanese Patent KOKOKU No. 57-28277, base polymers acid polymers, alkali metal or or alkaline earth metal salts of the acid polymers usable for the integral multilayer analytical element described in Japanese Patent KOKAI Nos. 59-143959 or 60-10171 and mixtures thereof are also usable as the above buffer.

Among the above pH buffers, examples of particulary preferable ones are a combination of disodium hydrogen phosphate, 3-morpholinoprepanesulfonic acid (MOPS, CAS Reg. No. [1132-61-2]) and sodium hydroxide, a combination of potassium dihydrogen phosphate and disodium hydrogen phosphate, a combination of disodium hydrogen phosphate and citric acid, a combination of boric acid, sodium chloride and borax, a combination of potassium dihydrogen phosphate and sodium tetraborate and the like.

The endogeneous ammonia-trapping layer is composed of reagents, such as, the above endogeneous ammonia-trapping reagent and the pH buffer and a hydrophilic polymer binder having a film-forming ability. As the hydrophilic polymer binder, the same hydrophilic polymer binder usable for the reagent layer can be used. Preferable hydrophilic polymer binders are, in general, gelatin, gelatin derivatives, hydroxyalkyl celluloses, polyvinyl alcohol and agarose.

The thickness of the endogeneous ammonia-trapping layer is usually about 1 to 30 μm, preferably about 2 to 10 μm.

The endogeneous ammonia-trapping layer preferably contains NADPH or NADH, -KG and GlDH. A preferred content (per 1 m^2 of the endogeneous ammonia-trapping layer) of these components is described below.

	Content ($/m^2$)
NADPH or NADH	80–7,000 mg
α-KG	100–10,000 mg
GlDH	2,000–100,000 units

In the case that the endogeneous ammonia-trapping layer contains aspartase and fumaric acid or fumarate, the content of aspartase is not less than 1,000 units/m^2, and the content of fumaric acid and/or fumarate is not less than 200 mg/m^2.

The endogeneous ammonia-trapping layer may be a microporous layer containing the ammonia-trapping reagent, the pH buffer and optional hydrophilic polymer provided in the porous spreading layer or between the porous spreading layer and the ammonia diffusion-hindering layer, as well as the substantially nonporous layer containing the polymer binder provided on the ammonia diffusion-hindering layer as mentioned previously. In the embodiment that the porous spreading layer contains the ammonia-trapping reagents, the spreading layer functions also as the endogeneous ammonia-trapping layer. In the microporous ammonia-trapping layer, the content (coating amount) of the ammonia-trapping reagent, the kind, content (coating amount) and pH range of the pH buffer and the like are substantially the same as the case of the nonporous layer. The adhesion of the endogeneous ammonia-trapping layer to the porous spreading layer is preferably conducted according to the adhesion technics of porous materials described in Japanese Patent KOKAI No. 61(1986)-4959(EP 0 166 365 A).

In the case of the analytical element for the determination of ammonia, it is a matter of course that the endogeneous ammonia-trapping layer is not provided.

A light-blocking layer may be provided beween the barrier layer and the endogeneous ammonia-trapping layer. The light-blocking layer is water-transmissive or water-permeable, and light-shielding particulates or particulates having both functions of light-shielding and light-reflecting are dispersed in and held by a small amount of a hydrophilic or weakly hydrophilic polymer binder having a film-forming ability. The light-blocking layer shields the color of an aqueous liquid sample, particularly the red color of hemoglobin in a whole blood sample, spotted on the spreading layer during measuring the detectable change which occurred in the indicator layer, such as, color change or color buffer, from the side of the light-transmissive support. This layer may also fuction as a light-reflecting layer or a background layer.

Examples of particulates having both functions of light-shielding and light-reflecting are titanium dioxide particulates, barium sulfate particulates, aluminum particulates and microflakes, and the like.

As the examples of the hydrophilic or weakly hydrophilic polymer binder having a film-forming ability, there are gelatin, gelatin derivatives, hydroxyalkyl cellulose, agarose, polyvinyl alcohol and the like. A known curing agent (crosslinking agent) may be blended with gelatin or gelatin derivatives.

The volume ratio of light-blocking particulates to hydrophilic polymer binder in the dry state is 10: about 2.5 to about 7.5, preferably about 3.0 to about 6.5. When the light-shielding particulates are titanium dioxide particulates, the ratio by weight of polymer binder is about 0.6 to about 1.8, preferably about 0.8 to 1.5 per 10 of titanium dioxide. The thickness of the light-blocking layer in the dry state is about 3 μm to about 30 μm, preferably about 5 μm to about 20 μm.

The porous spreading layer may be a woven fabric spreading layer disclosed in U.S. Pat. No. 4,292,272, U.S. Pat. No. 4,783,315, etc., such as, plain weaves including broad cloth and poplin, a knitted fabric spreading layer disclosed in EP 0 162 302 A, etc., such as tricot, double tricot or milanese, the spreading layer made of organic polymer fiber pulp-containing paper disclosed in Japanese Patent KOKAI No. 57-148250, a nonfibrous isotropic porous spreading layer, such as, a membrane filter (blushed polymer layer) disclosed in U.S. Pat. No. 3,992,158, a continuous microspaces-containing porous layers where polymer particulates, glass particulates or diatomaceous earth are dispersed in a hydrophilic polymer binder, or a continuous microspaces-containing porous layer where polymer particulates are joined so as to contact with each other at a point by using a polymer adhesive which does not swell

in water (three-dimensional lattice structure layer), or the like.

The porous spreading layer may function as the endogeneous ammonia-trapping layer by incorporating the ammonia-trapping reagent, pH buffer, etc. In this case, preferred spreading layers are fibrous spreading layers represented by woven fabric spreading layers and knitted fabric spreading layers in view that the reagent composition is easily incorporated therein.

An example of the method of incorporating the reagent composition containing the ammonia-trapping enzyme into the spreading layer is comprised of providing a porous spreading layer on a coating layer and then applying an aqueous solution or organic solvent-containing solution containing the reagent composition containing the enzyme onto the spreading layer, as disclosed in EP 0 119 861 A, EP 0 162 302 A, EP 0 162 301 A, etc.

Physical activation treatment represented by glow discharge or corona discharge disclosed in U.S. Pat. No. 4,783,315 may be provided on at least one side of the woven fabric or knitted fabric used as the porous spreading layer. The woven fabric or knitted fabric may be treated with degreasing by washing with water, or impregnating with a hydrophilic polymer. By providing the fabric with one or more of the above treatments, the fabric is rendered hydrophilic, and the adhesive force to the layer located on the underside, i.e. near the support, can be increased.

Although the endogeneous ammonia-trapping layer which is substantially nonporous and contains a hydrophilic polymer binder can function as an adhesive layer for joining the porous spreading layer directly without providing an adhesive layer separately, a known adhesive layer which is formed of a hydrophilic polymer represented by gelatin may be provided for the purpose of joining the spreading layer tightly. The thickness of the adhesive layer in a dry state is in the range of about 0.5 μm to 5 μm.

A surfactant may be incorporated in the indicator layer, the reagent layer, the ammonia diffusion-hindering layer, the endogeneous ammonia-trapping layer, the light-reflecting layer, the adhesive layer, the spreading layer containing or not containing the ammonia-trapping reagent composition, etc. A suitable surfactant is a nonionic surfactant, such as, p-octylphenoxypolyethyoxyethanol, p-nonylphenoxypolyethoxyethanol, polyoxyethylene oleyl ether, polyoxyethylenesorbitanmonolaurate, p-nonylphenoxypolyglycidol, octylglucoside, etc. The spreading action (metering action) for spreading an aqueous liquid sample is improved by adding a nonionic surfactant to the spreading layer. The water in an aqueous liquid sample is easily substantially uniformly absorbed by the reagent layer or a water absorption layer by adding a nonionic surfactant to these layers during analytical operations, and the liquid contact with the spreading layer becomes rapid and substantially uniform.

The multilayer analytical element of the invention can be prepared according to a known method disclosed in the specifications of the foregoing patents.

In view of manufacturing, packaging, transportation, storage, measuring operations, etc., the multilayer analytical element of the invention is preferably cut into square or circular pieces having a side or diameter of about 15 mm to about 30 mm, and put in a slide frame as disclosed in Jaapnese Patent KOKAI No. 57-63452, U.S. Pat. No. 4,169,751, U.S. Pat. No. 4,387,990, PCT application WO 83/00391, etc. for use. According to the object of use, the element may be put in a cassette or a magazine in the form of a strip, may be adhered to or put in a card having an opening, or the like.

The analysis of the analyte (ammonia-producing substance) in a liquid sample using the analytical element of the invention can be conducted according to the operations disclosed in the specifications of the foregoing patents. That is, about 5 μl to about 30 μl, preferably about 8 μl to about 15 μl of a drop of an aqueous liquid sample, such as, whole blood, blood plasma, blood serum or urine, is spotted onto the spreading layer, and incubated at a substantially constant temperature in the range of about 20° C. to about 400° C., preferably around 370° C., for 1 minute to 10 minutes. Then, the coloring or discoloration occurred in the element is measured by reflection photometry using a visible or ultraviolet light having a maximum absorption wave length or the vicinity thereof from the side of the light-transmissive support, and the content of the analyte in the liquid sample is determined by the principle of colorimetry using a calibration curve which was previously prepared. Alternatively, the fluorescence intensity emitted from the element is measured, and the content of the analyte in the liquid sample is determined by using a calibration curve which was previously prepared. By fixing the amount of the spotted liquid sample, incubation time and temperature, the quantitative analysis of an analyte can be conducted at a high accuracy. When the chemical analyzer disclosed in Japanese Patent KOKAI Nos. 60-125543, 60-220862, 61-294367, 58-161867, etc. is used, the quantitative analysis can be conducted by very simple operations at a high accuracy.

The analytical element of the invention is characterized by the binder polymer of the indicator layer and the polymer of the undercoat layer of the support, and the effects of the invention can be obtained by employing one of them.

The conventional element using the latex of vinyl acetate-acrylate ester copolymer as the binder for the indicator has problems of insufficient sensitivity, uneven coating due to the precipitation because of latex, unsuitable for the system using a pH indicator because of the occurrence of pH variation. However, all of the above problems can be resolved by employing a polyvinylalkyl ether as the binder.

On the other hand, in general, gelatin was undercoated onto the support of conventional element. When the indicator layer of the polyvinylalkyl ether is incorporated, a very small amount of ammonia contained in the gelatin is detected due to the improved sensitiity, and affects the background optical density adversely. Then, a binder polymer not containing ammonia is substituted for the gelatin undercoating for improving the adhesion of the indicator layer, and as a result, the background optical density is lowered. The measuring accuracy is further improved by the stabilization of the base line.

EXAMPLES

EXAMPLE 1

The following undercoat layer was coated onto a colorless transparent polyethylene terephthalate (PET) film having a thickness of 180 μm, and dried.

Undercoat Layer	
Gelatin	0.07 g/m^2
p-Chlorophenol	0.7 g/m^2

On the undercoat layer, the following indicator layer was applyed in a form of an ethanol solution, and dried.

Indicator Layer	
Bromophenol Blue	140 mg/m^2
Polyvinyl ethyl ether	2.1 g/m^2
(Weight average molecular weight: about 40,000)	
Sodium hydroxide	200 mg/m^2

Subsequently, on the indicator layer, a membrane filter made of polyethylene having a mean pore size of 0.2 μm, a void content of 75% and a thickness of 100 μm was uniformly pressed to provide an air barrier layer. On the air barrier layer, the following reagent layer was applied in a form of an aqueous solution, and dried.

Reagent Layer		
Hydroxyethyl cellulose		16 g/m^2
(Mean molecular weight: about 40,000)		
(Mean substitution degree of hydroxyethyl group:	1.0-1.3)	
(Average number of moles:	1.8-2.5)	
Sodium tetraborate		4 g/m^2
pH of the coating solution:		10.0

The above reagent layer was almost uniformly wetted with 0.2% p-nonylphenoxypolyglycidol aqueous solution. Immediately, a knitted fabric (gauge number: 40, 25% weight loss by aqueous NaOH solution treatment) was superposed onto the reagent layer, and laminated uniformly by passing between press rolls.

An ethanol solution of the following polyvinylpyrrolidon was impregnated into the laminate by applying for the purpose of improving spreading property, and dried to complete an integral multilayer analytical element for the determination of ammonia.

Polyvinylpyrrolidone	7.76 g/m^2
(Mean molecular weight: about 1,200,000)	

EXAMPLE 2

The following undercoat layer was coated onto a colorless transparent PET film having a thickness of 180 μm, and dried.

Undercoat Layer	
Polyvinyl methyl ether	0.035 g/m^2
(Weight average molecular weight: about 40,000)	
p-Chlorophenol	0.7 g/m^2

On the undercoat layer, the following indicator layer was applied in a form of an aqueous solution, and dried.

Indicator Layer	
Bromophenol Blue	340 mg/m^2
Vinyl acetate-ethylacrylate copolymer latex	8.5 g/m^2
N-Polyoxyethylene-N-octanesulfonamide	100 mg/m^2

Subsequently, on the indicator layer, a membrane filter made of polyethylene having a mean pore size of 0.2 μm, a void content of 75% and a thickness of 100 μm was uniformly pressed to provide an air barrier layer. On the air barrier layer, the following reagent layer was applied in a form of an aqueous solution, and dried.

Reagent Layer		
Hydroxyethyl cellulose		16 g/m^2
(Mean molecular weight: about 40,000)		
(Mean substitution degree of hydroxyethyl group:	1.0-1.3)	
(Average number of moles:	1.8-2.5)	
Sodium tetraborate		4 g/m^2
pH of the coating solution:		10.0

The above reagent layer was almost uniformly wetted with 0.2% p-nonylphenoxypolyglycidol aqueous solution. Immediately, a knitted fabric (gauge number: 40, 25% weight loss by aqueous NaOH solution treatment) was superposed onto the reagent layer, and laminated uniformly by passing between press rolls.

An ethanol solution of the following polyvinylpyrrolidone was impregnated into the laminate by applying for the purpose of improving spreading property, and dried to complete an integral multilayer analytical element for the determination of ammonia.

Polyvinylpyrrolidone	7.76 g/m^2
(Mean molecular weight: about 1,200,000)	

EXAMPLE 3

The following undercoat layer was coated onto a colorless transparent PET film having a thickness of 180 μm, and dried.

Undercoat Layer	
Polystyrene	0.035 g/m^2
p-Chlorophenol	0.7 g/m^2

On the undercoat layer, the following indicator layer was applyed in a form of an ethanol solution, and dried.

Indicator Layer	
Bromophenol Blue	340 mg/m^2
Vinyl acetate-ethylacrylate copolyer latex	8.5 g/m^2
N-Polyoxyethylene-N-octanesulfonamide	100 mg/m^2

Subsequently, on the indicator layer, a membrane filter made of polyethylene having a mean pore size of 0.2 μm, a void content of 75% and a thickness of 100 μm was uniformly pressed to provide an air barrier layer. On the air barrier layer, the following reagent layer was applied in a form of an aqueous solution, and dried.

Regent Layer	
Hydroxyethyl cellulose	16 g/m²
(Mean molecular weight: about 40,000)	
Mean substitution degree of hydroxyethyl group:	1.0-1.3
Average number of moles:	1.8-2.5
Sodium tetraborate	4 g/m²
pH of the coating solution:	10.0

The above reagent layer was almost uniformly wetted with 0.2% p-nonylphenoxypolyglycidol aqueous solution. Immediately, a knitted fabric (gauge number: 40, 25% weight loss by aqueous NaOH solution treatment) was superposed onto the reagent layer, and laminated uniformly by passing between press rolls.

An ethanol solution of the following polyvinylpyrrolidon was impregnated into the laminate by applying for the purpose of improving spreading property, and dried to complete an integral multilayer analytical element for the determination of ammonia.

Polyvinylpyrrolidone (Mean molecular weight: about 1,200,000)	7.76 g/m²

EXAMPLE 4

The following undercoat layer was coated onto a colorless transparent PET film having a thickness of 180 μm, and dried.

Undercoat Layer	
Polyvinyl methyl ether	0.035 g/m²
(Weight average molecular weight: about 40,000)	
p-Chlorophenol	0.7 g/m²

On the undercoat layer, the following indicator layer was applied in a form of an ethanol solution, and dried.

Indicator Layer	
Bromophenol Blue	140 mg/m²
Polyvinyl ethyl ether	2.1 g/m²
Sodium hydroxide	200 mg/m²

Subsequently, on the indicator layer, a membrane filter made of polyethylene having a mean pore size of 0.2 μm, a void content of 75% and a thickness of 100 μm was uniformly pressed to provide an air barrier layer. On the air barrier layer, the following reagent layer was applied in a form of an aqueous solution, and dried.

Reagent Layer	
Hydroxyethyl cellulose	16 g/m²
(Mean molecular weight: about 40,000)	
(Mean substitution degree of hydroxyethyl group:	1.0-1.3)
(Average number of moles:	1.8-2.5)
Sodium tetraborate	4 g/m²
pH of the coating solution:	10.0

The above reagent layer was almost uniformly wetted with 0.2% p-nonylphenoxypolyglycidol aqueous solution. Immediately, a knitted fabric (gauge number: 40, 25% weight loss by aqueous NaOH solution treatment) was superposed onto the reagent layer, and laminated uniformly by passing between press rolls.

An ethanol solution of the following polyvinylpyrrolidon was impregnated into the laminate by applying for the purpose of improving spreading property, and dried to complete an integral multilayer analytical element for the determination of ammonia.

Polyvinylpyrrolidone (Mean molecular weight: about 1,200,000)	7.76 g/m²

COMPARATIVE EXAMPLE 1

An integral multilayer analytical element for the determination of ammonia was prepared similar to Example 1, except that the indicator layer was changed to the following layer.

Indicator Layer	
Bromophenol Blue	340 mg/m²
Vinyl acetate-ethylacrylate copolyme latex	8.5 g/m²
N-Polyoxyethylene-N-octanesulfonamide	100 mg/m²

Evaluation of Quantitative Ability for Ammonia Determination

The integral multilayer analytical elements for the determination of ammonia of Examples 1 to 4 and Comparative Example 1 were evaluated by the following method.

Whole blood samples for the evaluation test were prepared by adding ammonium sulfate to a human whole blood so that the final concentration became about 50, 100, 150, 200, 250, 500 μg/dl. 10 μl of deminneralized distilled water or the whole blood sample for the evaluation test was spotted onto the spreading layer of each analytical element, and coloring optical density after 6 minutes was measured at 600 nm by the reflection photometry. Moreover, a calibration curve was prepared by using the above optical density values and the values obtained by measuring respective whole blood samples for the evaluation test by the glutamate dehydrogenase (GlDH) method. Besides, the above coloring test was repeated ten times as to each analytical element, and respective optical densities were measured. Each optical density was converted to ammonia concentration by using the above calibration curve, and variation coefficients were determined. The results of the analytical elements of Examples 1 and 4 and Comparative Example 1 are shown in Table 1, and the results of the analytical elements of Examples 2, 3, 4 and Comparative Example 1 are shown in Table 2.

As shown in Table 1, the coloring optical densities of the analytical elements of Examples I and 4 are higher than those of Comparative Example 1 were the polymer in the indicator layer is different, and moreover, the measuring accuracy is also improved.

As shown in Table 2, the reflection optical densities (background coloring optical density) of the analytical elements of Examples 2-4 obtained by spotting deminneralized distilled water are lower than those of Comparative Example 1 where the undercoating of the support is gelatin, and moreover, the measuring accuracy is also improved.

TABLE 1

	Analytical Element					
	Example 1		Example 4		Comparative 1	
Ammonia Conc. (μg/dl)	Optical Density (OD)	Variation Coeff. (CV)	Optical Density (OD)	Variation Coeff. (CV)	Optical Density (OD)	Variation Coeff. (CV)
0	0.32	—	0.14	—	0.32	—
52	0.45	6.4	0.30	4.4	0.34	17.4
87	0.52	4.0	0.37	2.8	0.41	9.8
134	0.65	2.4	0.49	2.1	0.51	7.5
186	0.72	1.5	0.58	1.6	0.60	6.0
233	0.85	2.3	0.65	1.0	0.66	5.1
484	1.11	2.0	1.02	1.6	0.92	4.7

TABLE 2

Ammonia	Analytical Element							
Conc.	Example 2		Example 3		Example 4		Comprative 1	
(μg/dl)	OD	CV	OD	CV	OD	CV	OD	CV
0	0.12	—	0.20	—	0.14	—	0.32	—
52	0.22	6.8	0.28	4.8	0.30	4.4	0.34	17.4
87	0.34	5.2	0.33	3.0	0.37	2.8	0.41	9.8
134	0.43	4.1	0.44	1.9	0.49	2.1	0.51	7.5
186	0.51	3.5	0.52	2.2	0.58	1.6	0.60	6.0
233	0.62	3.2	0.60	3.3	0.65	1.0	0.66	5.1
484	0.95	2.5	0.96	2.2	1.02	1.6	0.92	4.7

EXAMPLE 5

The following undercoat layer was coated onto a colorless transparent polyethylene terephthalate (PET) film having a thickness of 180)Am, and dried.

Undercoat Layer	
Gelatin	0.07 g/m^2
p-Chlorophenol	0.7 g/m^2

On the undercoat layer, the following indicator layer was applied in a form of an ethanol solution, and dried.

Indicator Layer	
Bromophenol Blue	140 mg/m^2
Polyvinyl ethyl ether	2.1 g/m^2
(Weight average molecular weight: about 40,000)	
Sodium hydroxide	200 mg/m^2

Subsequently, on the indicator layer, a membrane filter made of polyethylene having a mean pore size of 0.2 μm, a void content of 75% and a thickness of 100 μm was uniformly pressed to provide an air barrier layer. On the air barrier layer, the following reagent layer, the intermediate layer and the endogeneous ammonia-trapping layers were successively applied in a form of an aqueous solution, and dried.

Reagent Layer	
Alkali treated gelatin	11.7 g/m^2
Sodium tetraborate	1.7 g/m^2
p-Nonylphenoxypolyglycidol	300 mg/m^2
(Glycidol units: 10 on average)	
Creatinine iminohydrolase	750 U/m^2
(EC 3.5.4.21)	
pH of the coating solution: 8.0	
Ammonia Diffusion-Hindering Layer	
Alkali-treated gelatin	8.3 g/m^2
Sodium tetraborate	750 mg/m^2
p-Nonylphenoxypolyglycidol	200 mg/m^2

-continued

(Glycidol units: 10 on average)	
pH of the coating solution: 9.0	
Endogeneous Ammonia-Trapping Layer	
Alkali-treated gelatin	7.5 g/m^2
Sodium tetraborate	1.35 g/m^2
p-Nolylphenoxypolyglycidol	170 mg/m^2
(Glycidol units: 10 on average)	
α-Ketoglutaric acid	2.5 g/m^2
NADPH	1.6 g/m^2
Glutamate dehydrogenase	70,000 U/m^2
pH of the coating solution: 8.0	

The above endogeneous ammonia-trapping layer was almost uniformly wetted with 0.2% p-nonylphenoxypolyglycidol aqueous solution. Immediately, a knitted fabric (gauge number: 40, 25% weight loss by aqueous NaOH solution treatment) was superposed onto the reagent layer, and laminated uniformly by passing between press rolls.

An ethanol solution of the following polyvinylpyrrolidon was impregnated into the laminate by applying for the purpose of improving spreading property, and dried to complete an integral multilayer analytical element for the determination of creatinine.

Polyvinylpyrrolidone	7.76 g/m^2
(Mean moleculr weight: about 1,200,000)	

EXAMPLE 6

The following undercoat layer was coated onto a colorless transparent PET film having a thickness of 180 μm, and dried.

Undercoat Layer	
Polyvinyl methyl ether	0.035 g/m^2
(Weight average molecular weight: about 40,000)	
p-Chlorophenol	0.7 g/m^2

On the undercoat layer, the following indicator layer was applyed, and dried.

Indicator Layer	
Bromophenol Blue	340 mg/m^2
Vinyl acetate-ethylacrylate copolymer latex	8.5 g/m^2
N-Polyoxyethylene-N-octanesulfonamide	100 mg/m^2

Subsequently, on the indicator layer, a membrane filter made of polyethylene having a mean pore size of 0.2 μm, a void content of 75% and a thickness of 100 μm was uniformly pressed to provide an air barrier layer. On the air barrier layer, the following reagent layer, the intermediate layer and the endogeneous ammonia-trapping layers were successively applied in a form of an aqueous solution, and dried.

Reagent Layer	
Alkali-treated gelatin	11.7 g/m^2
Sodium tetraborate	1.7 g/m^2
p-Nonylphenoxypolyglycidol	300 mg/m^2
(Glycidol units: 10 on average)	
Creatinine iminohydrolase	750 U/m^2
(EC 3.5.4.21)	
pH of the coating solution: 8.0	
Ammonia Diffusion-Hindering Layer	
Alkali-treated gelatin	8.3 g/m^2
Sodium tetraborate	750 mg/m^2

-continued

p-Nonylphenoxypolyglycidol (Glycidol units: 10 on average)	200 mg/m^2
pH of the coating solution: 9.0	
Endogeneous Ammonia-Trapping Layer	
Alkali-treated gelatin	7.5 g/m^2
Sodium tetraborate	1.35 g/m^2
p-Nolylphenoxypolyglycidol (Glycidol units: 10 on average)	170 mg/m^2
α-Ketoglutaric acid	2.5 g/m^2
NADPH	1.6 g/m^2
Glutamate dehydrogenase	70,000 U/m^2
pH of the coating solution: 8.0	

The above endogeneous ammonia-trapping layer was almost uniformly wetted with 0.2% p-nonylphenoxypolyglycidol aqueous solution. Immediately, a knitted fabric (gauge number: 40, 25% weight loss by aqueous NaOH solution treatment) was superposed onto the reagent layer, and laminated uniformly by passing between press rolls.

An ethanol solution of the following polyvinylpyrrolidon was impregnated into the laminate by applying for the purpose of improving spreading property, and dried to complete an integral multilayer analytical element for the determination of creatinine.

Polyvinylpyrrolidone (Mean molecular weight: about 1,200,000)	7.76 g/m^2

EXAMPLE 7

The following undercoat layer was coated onto a colorless transparent PET film having a thickness of 180 μm, and dried.

Undercoat Layer	
Polystyrene	0.035 g/m^2
p-Chlorophenol	0.7 g/m^2.

On the undercoat layer, the following indicator layer was applyed in a form of an ethanol solution, and dried.

Indicator Layer	
Bromophenol Blue	340 mg/m^2
Vinyl acetate-ethylacrylate copolymer latex	8.5 g/m^2
N-Polyoxyethylene-N-octanesulfonamide	100 mg/m^2

Subsequently, on the indicator layer, a membrane filter made of polyethylene having a mean pore size of 0.2 μm, a void content of 75% and a thickness of 100 μm was uniformly pressed to provide an air barrier layer. On the air barrier layer, the following reagent layer, the intermediate layer and the endogeneous ammonia-trapping layers were successively applied in a form of an aqueous solution, and dried.

Reagent Layer	
Alkali-treated gelatin	11.7 g/m^2
Sodium tetraborate	1.7 g/m^2
p-Nonylphenoxypolyglycidol (Glycidol units: 10 on average)	300 mg/m^2
Creatinine iminohydrolase (EC 3.5.4.21)	750 U/m^2
pH of the coating solution: 8.0	

-continued

Ammonia Diffusion-Hindering Layer	
Alkali-treated gelatin	8.3 g/m^2
Sodium tetraborate	750 mg/m^2
p-Nonylphenoxypolyglycidol (Glycidol units: 10 on average)	200 mg/m^2
pH of the coating solution: 9.0	
Endogeneous Ammonia-Trapping Layer	
Alkali-treated gelatin	7.5 g/m^2
Sodium tetraborate	1.35 g/m^2
p-Nolylphenoxypolyglycidol (Glycidol units: 10 on average)	170 mg/m^2
α-Ketoglutaric acid	2.5 g/m^2
NADPH	1.6 g/m^2
Glutamate dehydrogenase	70,000 U/m^2
pH of the coating solution: 8.0	

The above endogeneous ammonia-trapping layer was almost uniformly wetted with 0.2% p-nonylphenoxypolyglycidol aqueous solution. Immediately, a knitted fabric (gauge number: 40, 25% weight loss by aqueous NaOH solution treatment) was superposed onto the reagent layer, and laminated uniformly by passing between press rolls.

An ethanol solution of the following polyvinylpyrrolidon was impregnated into the laminate by applying for the purpose of improving spreading property, and dried to complete an integral multilayer analytical element for the determination of creatinine.

Polyvinylpyrrolidone (Mean molecular weight: about 1,200,000)	7.76 g/m^2

EXAMPLE 8

The following undercoat layer was coated onto a colorless transparent PET film having a thickness of 180 μm, and dried.

Undercoat Layer	
Polyvinyl methyl ether (Weight average molecular weight: about 40,000)	0.035 g/m^2
p-Chlorophenol	0.7 g/m^2

On the undercoat layer, the following indicator layer was applyed, and dried.

Indicator Layer	
Bromophenol Blue	140 mg/m^2
Polyvinyl ethyl ether (Weight average molecular weight: about 40,000)	2.1 g/m^2
Sodium hydroxide	200 mg/m^2

Subsequently, on the indicator layer, a membrane filter made of polyethylene having a mean pore size of 0.2 μm, a void content of 75% and a thickness of 100 μm was uniformly pressed to provide an air barrier layer. On the air barrier layer, the following reagent layer, the intermediate layer and the endogeneous ammonia-trapping layers were successively applied in a form of an aqueous solution, and dried.

Reagent Layer	
Alkali-treated gelatin	11.7 g/m^2
Sodium tetraborate	1.7 g/m^2
p-Nonylphenoxypolyglycidol (Glycidol units: 10 on average)	300 mg/m^2

-continued

Creatinine iminohydrolase (EC 3.5.4.21)	750 U/m^2
pH of the coating solution: 8.0	
Ammonia Diffusion-Hindering Layer	
Alkali-treated gelatin	8.3 g/m^2
Sodium tetraborate	750 mg/m^2
p-Nonylphenoxypolyglycidol (Glycidol units: 10 on average)	200 mg/m^2
pH of the coating solution: 9.0	
Endogeneous Ammonia-Trapping Layer	
Alkali-treated gelatin	7.5 g/m^2
Sodium tetraborate	1.35 g/m^2
p-Nolylphenoxypolyglycidol (Glycidol units: 10 on average)	170 mg/m^2
α-Ketoglutaric acid	2.5 g/m2
NADPH	1.6 g/m^2
Glutamate dehydrogenase	70,000 U/m^2
pH of the coating solution: 8.0	

The above endogeneous ammonia-trapping layer was almost uniformly wetted with 0.2% p-nonylphenoxypolyglycidol aqueous solution. Immediately, a knitted fabric (gauge number: 40, 25% weight loss by aqueous NaOH solution treatment) was superposed onto the reagent layer, and laminated uniformly by passing between press rolls.

An ethanol solution of the following polyvinylpyrrolidon was impregnated into the laminate by applying for the purpose of improving spreading property, and dried to complete an integral multilayer analytical element for the determination of creatinine.

Polyvinylpyrrolidone (Mean molecular weight: about 1,200,000)	7.76 g/m^2

COMPARATIVE EXAMPLE 2

An integral multilayer analytical element for the determination of creatinine was prepared similar to Example 5, except that the indicator layer was changed to the following layer.

Indicator Layer	
Bromophenol Blue	340 mg/m^2
Vinyl acetate-ethylacrylate copolymer latex	8.5 g/m^2
N-Polyoxyethylene-N-octanesulfonamide	100 mg/m^2

Evaluation of Quantitative Ability for Creatinine Determination

The integral multilayer analytical elements for the determination of ammonia of Examples 5 to 8 and Comparative Example 2 were evaluated by the following method.

Whole blood samples for the evaluation test were prepared by adding creatinine to a human whole blood so that the final concentration became about 1.0, 4.0, 11.0 μg/dl. 10 μl of demineralized distilled water or the whole blood sample for the evaluation test was spotted onto the spreading layer of each analytical element, and coloring optical density after 6 minutes was measured at 600 nm by the reflection photometry. Moreover, a calibration curve was prepared by using the above optical density values and the values obtained by measuring respective whole blood samples for the evaluation test by the Jaffé method. Besides, the above coloring test was repeated ten times as to each analytical element, and respective optical densities were measured. Each optical density was converted to creatinine concentration by using the above calibration curve, and variation coeffcients were determined. The results of the analytical elements of Examples 5 and 8 and Comparative Example 2 are shown in Table 3, and the results of the analytical elements of examples 6, 7, 8 and Comprarative Example 2 are shown in Table 4.

As shown in Table 3, the coloring optical densities of the analytical elements of Examples 5 and 8 are higher than those of Comparative Example 2 where the polymer in the indicator layer is different, and moreover, the measuring accuracy is also improved.

As shown in Table 4, the reflection optical densities (background coloring optical density) of the analytical elements of Examples 6-8 obtained by spotting demineralized distilled water are lower than those of Comprative Example 2 where the undercoating of the support is gelatin, and moreover, the measuring accuracy is also improved.

TABLE 3

Creatinine Conc. (mg/dl)	Analytical Element: Example 5 Optical Density (OD)	Example 5 Variation Coeff. (CV)	Example 8 Optical Density (OD)	Example 8 Variation Coeff. (CV)	Comparative 2 Optical Density (OD)	Comparative 2 Variation Coeff. (CV)
0	0.28	—	0.16	—	0.32	—
1.2	0.51	0.7	0.39	0.6	0.40	6.8
4.1	0.76	0.6	0.64	0.5	0.58	6.2
10.9	1.10	0.8	1.02	0.6	0.81	4.2

TABLE 4

Creatinine Conc. (mg/dl)	Analytical Element: Example 6 OD	Example 6 CV	Example 7 OD	Example 7 CV	Example 8 OD	Example 8 CV	Comprative 1 OD	Comprative 1 CV
0	0.12	—	0.20	—	0.16	—	0.32	—
1.2	0.30	2.0	0.36	1.8	0.39	0.6	0.40	6.8
4.1	0.48	1.3	0.60	1.6	0.64	0.5	0.58	6.2
10.9	0.76	1.6	1.00	0.8	1.02	0.6	0.81	4.2

We claim:

1. In an integral multilayer analytical element for the determination of ammonia or an ammonia-producing substance comprising a light-transmissive liquid-impermeable support, an indicator layer containing an indicator which produces a detectable change by gaseous ammonia, a liquid permeation barrier layer, a reagent layer containing an alkaline buffer and optionally a reagent capable of reacting with said ammonia-producing substance to produce ammonia, and, a spreading layer, laminated in this order, the improvement which comprises that the indicator layer contains a polyvinyl alkyl ether as a binder polymer.

2. The analytical element of claim 1 wherein said polyvinyl alkyl ether is a member selected from the group consisting of polyvinyl methyl ether, polyvinyl ethyl ether and polyvinyl isobutyl ether.

3. In an integral multilayer analytical element for the determination of ammonia or an ammonia-producing substance comprising a light-transmissive liquid-impermeable support, an indicator layer containing an indicator which produces a detectable change by gaseous ammonia, a liquid permeation barrier layer, a reagent layer containing an alkaline buffer and optionally a reagent capable of reacting with said ammonia-producing substance to produce ammonia and a spreading

layer laminated in this order, the improvement which comprises that the surface of said support facing toward the indicator layer is undercoated with a polymer selected from the group consisting of a polyvinyl alkyl ether, a hydroxyalkyl cellulose, an alkyl cellulose, polystyrene, a polyalkyl methacrylate, polyvinylidene chloride, polyvinyl alcohol and polyvinyl pyrrolidone.

4. The analytical element of claim 1 wherein the surface of said support facing toward the indicator layer is undercoated with a polymer selected from the group consisting of a polyvinyl alkyl ether, a hydroxyalkyl cellulose, an alkyl cellulose, polystyrene, a polyalkyl methacrylate, polyvinylidene chloride, polyvinyl alcohol and polyvinyl pyrrolidone.

5. The analytical element of claim 3 or 4 wherein said surface is undercoated with polyvinyl alkyl ether.

6. The analytical element of claim 5 wherein said polyvinyl alkyl ether is a member selected from the group consisting of polyvinyl methyl ether, polyvinyl ethyl ether and polyvinyl isobutyl ether.

7. The analytical element of claim 1, 3 or 4 wherein said liquid permeation barrier layer is an air barrier layer composed of a porous material having continuous voids.

8. The analytical element of claim 7 wherein said porous material is a membrane filter made of a material selected from the group consisting of polyethylene, polypropylene and polytetrafluoroethylene.

9. The analytical element of claim 1, 3, or 4, which further comprises an ammonia diffusion-preventing layer comprising a polymer binder placed on the reagent layer.

10. The analytical element of claim 9 wherein said polymer binder is selected from the group consisting of hydroxyalkyl cellulose, agarose and polyvinyl alcohol.

11. The analytical element of claim 9 which is for the determination of an ammonia-producing substance and further comprises an endogeneous ammonia-trapping layer containing a reagent composition containing an enzyme of which the substrate is ammonia.

12. The analytical element of claim 1 wherein the indicator contains 1 to 2% by weight of the binder polymer.

13. The analytical element of claim 3 wherein the indicating layer is coated with 5 to 500 mg/m^2 of the polymer.

14. In an integral multilayer analytical element for the determination of ammonia or an ammonia-producing substance consisting essentially of a light-transmissive liquid-impermeable support, an indicator layer containing an indicator which produces a detectable change by gaseous ammonia, a liquid permeation barrier layer, a reagent layer containing an alkaline buffer and optionally a reagent capable of reacting with said ammonia-producing substance to produce ammonia, and, a spreading layer, laminated in this order, the improvement which comprises that the indicator layer contains a polyvinyl alkyl ether as a binder polymer.

* * * * *

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Analysis of single polymer beads for solid phase combinatorial synthesis: the determination of reactive thiol and within batch polydispersity of bead loadings

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Polymer beads are the starting point of many synthetic protocols, and in a number of combinatorial syntheses such studies are based on single beads. It has therefore become important that individual beads carry the same functional group activity and quality control of populations of beads requires a knowledge of the distribution of activity between the individual beads. A procedure was developed for the measurement of the thiol loading of single copolymer beads which is based on the bleaching of Michler's hydrol [4,4′-bis(dimethylamino)-diphenylcarbinol (BDC-OH)]. Flow injection colorimetry permits the small volumes of solution generated from single beads to be measured with rapid turnover and with a reproducibility of *ca.* 2%. The solution detection limit of 0.17 mM corresponds to a bead thiol concentration of 0.33 mmol g^{-1}. The procedure and the variability of a bead population were demonstrated using modified styrene–divinylbenzene copolymer beads. The apparatus allowed fast, simple and accurate determinations to be carried out on the individual polymer beads. Within a single batch of thiol-modified styrene–divinylbenzene beads thiol loadings ranged from <0.35 to 2.07 mmol g^{-1} or 0.12 to 1.3 μmol per bead. Polydispersity may therefore significantly influence screening decisions based on single bead syntheses.

Introduction

The methods of solid phase synthesis and combinatorial chemistry are now established tools in laboratory synthesis and are widely employed to generate vast compound libraries to be screened for activity. Whilst there are many alternative strategies which can be employed in combinatorial chemistry, the principal is well illustrated by the so-called 'mix and split protocol'.[1] In such synthetic routes a portion of a batch of beads resulting from a single solid phase synthesis is divided into aliquots. A number of alternative reaction steps are then carried out on each of these aliquots. The beads are then recombined, again randomly split into aliquots and subjected to further reaction steps. Such a sequence results in a large number of beads, each of which is attached to only one product. At this stage it is important that the yield of one product is not restricted by its association with a single bead having a low density of active sites. If this were to occur, screening of the beads for target activity might wrongly lead to the conclusion that a compound associated with a poor quality bead was inactive.

Loadings, often given by manufacturers, are an average value of the number of moles of active sites per mass of beads, yet how widely do beads vary within the batch, and how does this variation affect the compound library we obtain? Even if constant activity between beads can be ensured, it is necessary to have in place techniques that can be applied to assess the progress of subsequent reactions on the beads.

Colorimetric methods for the measurement of functional groups such as amines and thiols in solution are well established but the quantitative measurement of reactive groups within and on a solid are less well known. Those methods that have been presented have been limited to the measurement of bulk samples of solid, thereby measuring average loadings.

In developing quantitative colorimetric procedures for the analysis of solids, it is generally necessary to measure a coloured product that is no longer attached to the solid. Methods that result in the formation of coloured products linked to the bead result in quantification difficulties as the coloured product is distributed through the bead structure.

There are a number of different reagent systems that could be potentially employed to measure solid-bound thiol groups. These include the Ellman test,[2] *p*-hydroxymercuribenzoate,[3] *N*-ethylmaleimide[4] and Michler's hydrol. The last compound, 4,4′-bis(dimethylamino)diphenylcarbinol (BDC-OH), forms an intense blue carbonium–immonium ion under acidic conditions that is bleached by reaction with thiols. The molar absorptivity of the chromophore can be enhanced several-fold by the addition of guanidine hydrochloride.[5]

With the development of bead-based combinatorial chemistry has come a dependence on the characteristics of a single bead. It is therefore necessary for new methods to be developed which are able to determine functional group densities of individual beads and to monitor the progress of reactions on and within individual beads. The overall objective of this investigation was therefore to achieve an experimental protocol which could be employed to assess the thiol content of individual polymer beads based on the BDC colour chemistry. By employing flow injection analysis, and the modification of the colour chemistry for use with single beads, it has been possible to develop a simple and accurate method capable of analysing the low volumes of sample solution generated from single beads.

Experimental

Apparatus

When measuring the average thiol concentration of several milligrams of beads, the 2 mL of solution required for conventional colorimetric instrumentation can be readily employed. As the mass of bead material is decreased, however, either the volume of solution or the magnitude of the measured

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absorbance has to be adjusted to reflect the smaller mass of functional group that is available for measurement. To maintain the absorbance of the analyte solution high, small volumes of solution must be utilised for the analysis of a single bead. Flow injection apparatus is well suited to use with low volume sample solutions.

The apparatus employed in this work was constructed around a colorimeter system employing a low volume (10 μL, 1 cm pathlength) cell. Carrier solution of 60% ethanol–water was pumped through the system by a Varian 2010 HPLC pump at a flow rate of 1.2 mL min^{-1}. A Rheodyne Model 7125 loop injector was employed to introduce the reacted BDC solution into the carrier stream. The detector was an HPLC detector (Du Pont Instruments, Model 852001-902 UV spectrophotometer) modified for use at 600 nm by replacing the UV light source with a Pro-Lite Plus dichroic lamp (12 V, 50 W). A 470 nm longpass edge filter was incorporated in the light beam to cut out UV radiation. Absorbance was output on a Servogor 120 chart recorder. At the end of the solvent flow line a back-pressure regulator (Part No. 39020. Alltech, Carnforth, UK) was employed to control the formation of gas bubbles within the flow cell.

Bead preparation

Thiol-modified polymer beads were prepared from Amberlite XAD-4 (particle size 0.30–0.78 mm) (BDH, Poole, UK) using the method described by Phillips and Fritz.[6] Elemental sulfur analysis of the derivatised beads was carried out by the Microanalysis Laboratory of Imperial College, London.

Reagent solutions

Guanidine hydrochloride (GuHCl). A 2 M guanidine hydrochloride (Avocado, Heysham, UK) solution was prepared in 0.04 M sodium acetate (BDH) buffer (pH 5.1)–ethanol (2 + 3 v/v).

4,4′-Bis(dimethylamino)diphenylcarbinol solution (BDC-OH). A 6–7 mg amount of 4,4′-bis(dimethylamino)diphenylcarbinol (Sigma, Poole, UK) was dissolved in acetone (10 mL).

Thiol stock standard solution. The sodium salt of thioglycolic acid (8–9 mg) (Sigma) was weighed accurately and dissolved in the buffered GuHCl reagent (10 mL).

Performance

Two experiments were carried out to assess the combined reproducibility of solution preparation and absorbance measurement. The background high absorbance measurement was assessed by the analysis of 10 separate preparations of BDC solutions containing no added thiol. Aliquots of 100 μL of the BDC stock standard solution were placed in 10 mL calibrated flasks, which were then diluted to volume with GuHCl solution. After an equilibration period of 10 min at room temperature, each solution was measured three times. The reproducibility of measurements on thiol-containing samples was similarly assessed by the triplicate injection of 10 solutions, prepared as described previously, but with the addition of 1 mL of the thiol stock standard solution.

The detection limit of the method was determined by decreasing the thiol concentration of the solution until it was indistinguishable from the blank.

Development of the colorimetric procedure

Initial experiments with derivatised beads identified problems associated with the adsorption of the coloured cation on the bead surface from an aqueous BDC solution containing guanidinium hydrochloride, a problem similar to that which had previously been encountered in the development of a quantitative test for copolymer-supported amines.[7] Whilst the adsorbed colour reagent could be desorbed into a solution of tetraethylammonium chloride in dichloromethane,[7] this solvent was incompatible with the aqueous sodium acetate buffer employed in the original BDC thiol procedure.[5] The adsorption problem was overcome by adjusting the composition of the buffer solution to contain 60% ethanol. This also meant that the concentration of guanidine hydrochloride used had to be decreased, thereby reducing the molar absorptivity of the BDC cation. With a new buffer [0.04 M sodium acetate buffer (pH 5.1)–ethanol, containing 2 M guanidine hydrochloride (2 + 3)] the coloured cation did not adsorb on the bead surface, thereby obviating the need for a washing step.

The developed analytical protocol

Sample preparation. To an accurately weighed bead in a sample tube add GuHCl solution (0.5 mL) and BDC stock standard solution (10 μL) and mix. After 10 min, sonicate the mixture for 10 min in a sonic bath. Quantitatively transfer the sample solution into a 1 mL calibrated flask, leaving the bead in the sample tube. Add a second aliquot of GuHCl solution (0.2 mL) to the sample tube, sonicate for 5 min and quantitatively transfer the resulting solution to the calibrated flask and dilute to volume with GuHCl solution. These volumes can be reduced to cover the measurement of smaller beads.

The same procedure was scaled up for the analysis of 5–6 mg portions of the derivatised beads to assess their average thiol loading.

Calibration. Calibration was carried out by the addition of known volumes (0.5, 1.0, 1.5, 2.0 and 2.5 mL) of the thiol stock standard solution to 10 mL calibrated flasks containing 100 μL of the BDC stock standard solution. The resulting solutions were diluted to volume with GuHCl solution and then allowed to equilibrate at room temperature for 10 min before analysis.

Results and discussion

System performance

Output. With a typical injection spacing of 1–2 min, baseline resolution of the absorbance peaks was achieved (Fig. 1).

Reproducibility. The triplicate injection of 10 separately prepared thiol-free BDC solutions into the flow injection system

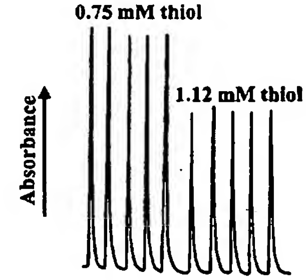

Fig. 1 Typical output trace from the flow injection system.

resulted in peak heights ranging from 59.3 to 64.3 mm, giving a mean of 61.67 mm with a pooled estimate of the standard deviation of 1.42 mm. This corresponds to a relative standard deviation (RSD) of 2.11%.

A second experiment was carried out in which 10 separately prepared BDC solutions containing 1 mL of the thiol stock standard solution were each injected into the system three times. The pooled relative standard deviation of the results was 1.32%. The improved precision with sample solutions containing added thiol is believed to result from the high solution absorbance of the thiol-free BDC solutions; as this is a technique which is based on the bleaching of the blue coloration of the BDC solution, the addition of thiol results in solutions of lower absorbance, that can be measured with higher precision.

Detection limit. The detection limit of the thiol determination method is 0.17 mM thiol; on a bead having a mass of 0.5130 mg this equates to a thiol loading of 0.33 mmol g^{-1}. This can be further reduced by the use of smaller reagent volumes and lower concentrations of BDC. A typical calibration graph is shown in Fig. 2.

The molar absorptivity of the BDC cation at 600 nm increases with increase in guanidine hydrochloride concentration.[5] Problems with the adsorption of the BDC cation on bead surfaces were overcome by dissolving the GuHCl in buffered 60% ethanol instead of the previously used aqueous solvent. A decrease in the guanidine hydrochloride concentration was necessary, however, and this degraded the molar absorptivity of the BDC. This sacrifice was considered necessary, however, to ensure the complete removal of the BDC from the bead surface. As the molar absorptivity of the BDC solution depends on the solvent composition it is important to ensure that the composition is maintained constant for all solutions to be measured.

Bead characteristics

The mass distribution of the beads measured in this study is shown in Fig. 3. The arithmetic mean bead mass was 0.5130 mg.

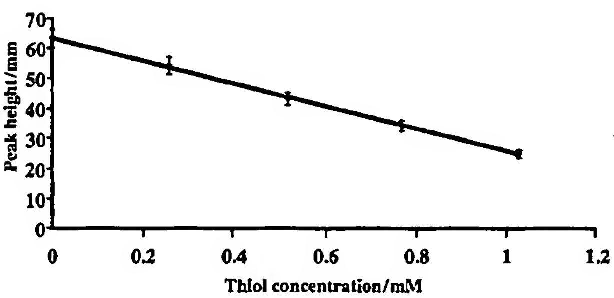

Fig. 2 Calibration curve.

Elemental sulfur analysis showed derivatised beads to contain 5.06% w/w sulfur, and underivatised beads 0.19% w/w sulfur. This equates to a thiol loading of approximately 1.52 mmol g^{-1} if it is assumed that the whole sulfur content of the beads is due to thiol groups. By batch colorimetric analysis the average thiol loading of the bulk copolymer beads was found to be 1.08 mmol g^{-1} of resin, with a relative standard deviation of 8% (n = 5). Discrepancies between these two measures of sulfur can arise for a number of reasons. First, for these batch sulfur analyses it was necessary to employ a small number of polymer beads, thereby leading to sampling problems. The two methods also measure two different analytical properties of the beads. The BDC procedure is selective towards the free active thiol group and would not detect, for example, thiol groups that had oxidised to disulfide. The procedure also only assesses those sulfide groups that are available for reaction, whereas the combustion based total sulfur measurement is non-selective and includes sulfur that is deeply included in the bead structure and inaccessible to reagents. It is therefore considered unlikely that total sulfur measurements are able to give an accurate reflection of the available thiol loading of a bead and that the colorimetric measure would be a truer indicator of sites that are available for synthetic coupling.

When single polymer beads were analysed, their concentration based thiol loadings were found to vary significantly, ranging from 0.25 to 2.07 mmol g^{-1} (Fig. 4).

The numerical average of the thiol concentrations of 100 individual beads was 0.95 mmol g^{-1} with an RSD of 34%. If these beads had been analysed in a single batch this would have corresponded to a calculated mean bead thiol loading of 0.92 mmol g^{-1}; a value that is not significantly different from the bulk measure of thiol (1.08 mmol g^{-1}) resulting from the analysis of a relatively small number of beads. Whilst the overall trend of the data presented in Fig. 4 follows the general characteristics of a normal distribution, it is evident that beads differ significantly in their thiol capacity when measured relative to the mass of the bead. This variability can be clearly seen from the individual analysis results (Fig. 5).

Not unexpectedly, the trend of the data is for the larger beads to show a higher mass loading of reactive groups per bead. It is the distribution of data about the trend line that illustrates most clearly the problems that might be encountered in the selection of individual beads. In the extreme, for this batch of beads, those lying in the mass range 0.6–0.8 mg, have thiol loadings lying between *ca.* 0.25 and 1.1 μmol per bead. This fourfold difference could significantly bias the interpretation of activity data obtained from individual beads. Such variability can arise for a variety of synthetic reasons, including surface inactivity due to residual salts from the synthesis of the bead itself, inclusion of air within the bead during its modification and partial collapse of the internal structure of the polymer by solvation or heating effects.

Whilst the technique has in this instance been applied only to the analysis of thiol loadings of derivatised copolymer beads, it

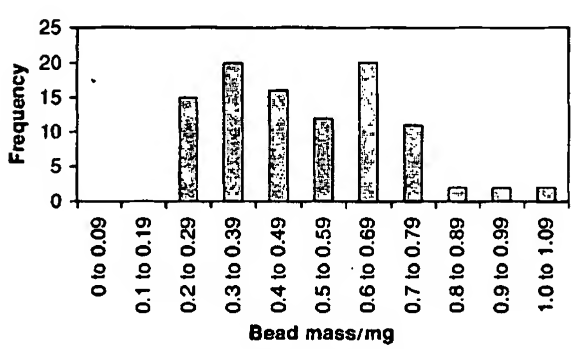

Fig. 3 Mass distribution of thiol beads.

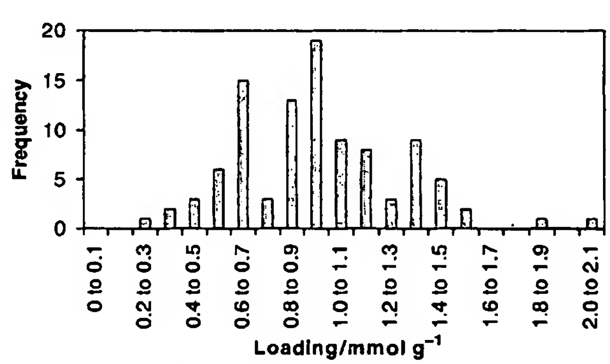

Fig. 4 Thiol concentration in beads.

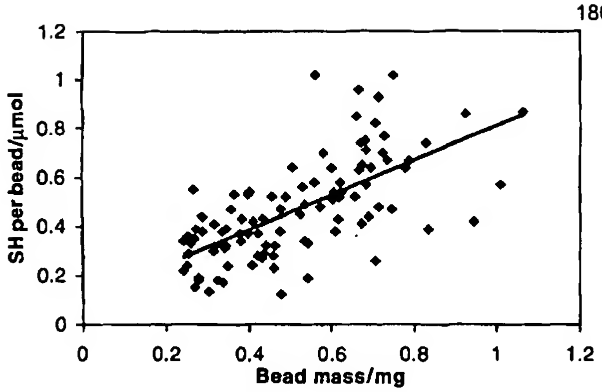

Fig. 5 Thiol content of individual beads shown on the basis of moles of thiol per bead.

should also prove a useful tool in the analysis of terminal thiol groups in solid phase combinatorial synthesis, allowing monitoring of reaction efficiency in synthetic routes involving thiol intermediates.

Conclusions

The procedure detailed in this paper provides a simple and effective tool for the analysis of single beads for combinatorial chemistry. Flow injection analysis has proved to be an effective approach to the measurement of the small sample volumes generated in the analysis of single polymer beads. When used with the thiol-sensitive BDC colorimetric reagent a reliable system has been developed for analysis of thiol-functionalised solid phase materials for solid phase/combinatorial synthesis. The approach was applied to the analysis of single polymer beads, demonstrating the variability of bead capacities and the potential influence that bead capacity might have on the interpretation of measurements employed in the screening of individual library members for activity. As bead synthesis technology advances, increasingly demanding applications of single bead combinatorial chemistry are developed and there will be an increasing need for the production of beads having both very narrow particle size distributions and consistent single bead loadings of reactive sites. This paper has demonstrated bead-to-bead variability of thiol loadings within a single batch of derivitised beads. Such variability could have a particularly significant impact on the interpretation of the activity of a compound associated with a low activity bead. Whilst this paper has focused on the application of the analytical procedure to the measurement of thiol loadings on individual polymer beads, it is equally applicable to the monitoring of reaction progress in solid phase syntheses involving the linking of thiol intermediates or products to the solid phase.

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DOCUMENT-IDENTIFIER: US 20020068364 A1
TITLE: Integral-multilayer analytical element for analysis of ammonia or ammonia-producing substance

Summary of Invention Paragraph:
[0002] Up to now, a variety of the so-called dry chemistry methods have been proposed in order to carry out the analysis of urea nitrogen in body fluids simply and rapidly without personal errors. A typical dry chemistry method uses an integral multilayer analytical element comprising a reagent layer containing urease and an alkaline buffering agent, an indicator layer for the detection of gaseous ammonia, and a selective permeation layer which is interposed between the reagent and the indicator layers and which permits only gaseous ammonia to pass therethrough.

Summary of Invention Paragraph:
[0011] On the support, an indicator layer is provided. The indicator layer contains one or more compounds which change in absorption wavelength as a result of the reaction with gaseous ammonia (hereinafter, the compound is referred to as a dye precursor). The dye precursor which may be used in the analytical element of this invention includes leuco dyes, such as leucocyanine dye, nitro-substituted leuco dye and leucophthalein dye described in U.S. Pat. No. Re. 30,267, pH indicators, such as Bromophenol Blue, Bromocresol Green, Bromothymol Blue, Qinoline Blue and rosolic acid (see "Kagaku Dai-Jiten" (Chemical Dictionary) Kyoritsu, vol. 10, 63-65), triarylmethane dye precursors, leucobenzilidene pigments (see JP 1982-145273 A), diazonium salts and azo dye couplers, base bleachable dyes, and the like.

Summary of Invention Paragraph:
[0020] On the liquid blocking layer, a reagent layer is provided. The reagent layer is the layer usually containing a reagent reacting with the ammonia-producing substance to produce ammonia (generally an enzyme or a reagent containing an enzyme), an alkaline buffering agent for efficiently releasing the ammonia produced during the reaction in a form of gaseous ammonia, and a hydrophilic polymer binder having a film-forming facility. Examples of combination of ammonia-producing substance/reagent are urea/urease, creatinine/creatinine deiminase, amino acid/amino acid dehydrogenase, amino acid/amino acid oxidase, amino acid/amino acid dehydratase, amino acid/ammonia lyase, amine/amine oxidase, diamine/amine oxidase, glucose and phosphoamidate/phosphoamidate hexose phosphotransferase, ADP/carbamate kinase and carbamoylphosphate, acid amide/amide hydrolase, nucleobase/deaminase, nucleoside/deaminase, nucleotide/deaminase, guanine/guanase, and the like.

Previous Doc Next Doc Go to Doc#

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(54) INTEGRAL-MULTILAYER ANALYTICAL ELEMENT FOR ANALYSIS OF AMMONIA OR AMMONIA-PRODUCING SUBSTANCE

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(52) **U.S. Cl.** **436/113**

(57) **ABSTRACT**

An integral multilayer analytical element for analysis of ammonia or ammonia-producing substance is provided, which comprises a thin liquid blocking layer having a comparative facility with a thicker liquid blocking layer and is stably fabricated on a common production line employed for different type of slides.

In the integral multilayer analytical element for analysis of ammonia or ammonia-producing substance comprising a transparent support, an indicator layer containing an indicator which produces a detectable change by gaseous ammonia, a liquid blocking layer permitting a gaseous ammonia to pass through, a reagent layer containing an alkaline buffering agent and optionally a reagent capable of reacting with said ammonia-producing substance to produce ammonia, and a spreading layer, adhesively laminated in this order, the improvement which comprises that said liquid blocking layer is composed of at least two porous membranes.

INTEGRAL-MULTILAYER ANALYTICAL ELEMENT FOR ANALYSIS OF AMMONIA OR AMMONIA-PRODUCING SUBSTANCE

FIELD OF THE INVENTION

[0001] The present invention relates to an integral multilayer analytical element for use in the analysis of ammonia or an ammonia-producing substance in liquid samples, and more particularly to an integral multilayer analytical element suitable for use in the analysis (assay) of ammonia or an ammonia-producing substance, such as creatinine, urea, etc., in body fluids, such as blood, urine, etc.

BACKGROUND OF THE INVENTION

[0002] Up to now, a variety of the so-called dry chemistry methods have been proposed in order to carry out the analysis of urea nitrogen in body fluids simply and rapidly without personal errors. A typical dry chemistry method uses an integral multilayer analytical element comprising a reagent layer containing urease and an alkaline buffering agent, an indicator layer for the detection of gaseous ammonia, and a selective permeation layer which is interposed between the reagent and the indicator layers and which permits only gaseous ammonia to pass therethrough.

[0003] For example, JP 1977-003488 A (Corresponding to U.S. Pat. No. Re. 30,267) discloses an integral analytical element having the fundamental multilayer structure described above. This analytical element uses a thin hydrophobic polymer layer as a selective permeation layer for gaseous ammonia.

[0004] JP 1983-077661 A discloses an integral multilayer analytical element for use in the analysis of ammonia or an ammonia-producing substance in liquid samples comprising a laminate of a transparent support, an indicator layer for gaseous ammonia, a liquid blocking layer, a reaction layer containing an alkaline buffering agent and a optionally reagent capable of producing ammonia by the reaction with the ammonia-producing substance, and a porous spreading layer in this order. The integral multilayer analytical element is characterized in that the liquid blocking layer is made of a porous substance comprising pores which function as air vents substantially cutting off liquid samples and permitting gaseous ammonia to pass therethrough under a condition of usage. In the multilayer analytical element, a membrane filter is used as a selectively transmissive layer to approve adhesion to the indicator layer and to give high sensitivity.

[0005] Further, JP 1992-157363 A discloses usage of polyvinyl alkyl ether etc. substantially free from ammonia and ammonium ion as an under coating on a support or a binder for an indicator layer to obtain an integral multilayer analytical element for use in the analysis of ammonia or an ammonia-producing substance in liquid samples with higher color development optical density, low color development optical density of background and higher measurement accuracy. JP 1992-157364 A discloses usage of a porous spreading layer containing poly-N-vinyl pyrrolidone and a binder for a reagent layer for an ammonia-producing reaction which does not contain substantially any ammonia, and does not generate ammonia or vary in its binder performance at a pH value of about 9 or more to obtain an integral multilayer analytical element for use in the analysis of ammonia or an ammonia-producing substance in liquid samples with higher color development optical density, low color development optical density of background and higher measurement accuracy.

[0006] Yet, the whole thickness of these conventional analytical slides is large since they have a thick porous membrane as a liquid blocking layer. Difference in thickness of these slides and other slides to analyze other analytes requiring no liquid blocking layer is so large that it is unstable to fabricate them on a same production line. However there is a limit in using a thinner porous membrane because of deterioration in its performance as a liquid blocker. Further, though there is a slide having a very thin liquid blocking layer using cellulose acetate butylate, an organic solvent is required to fabricate it, resulting in problems on equipments and environments.

SUMMARY OF THE INVENTION

[0007] The purpose of this invention is to provide an integral multilayer analytical element for use in the analysis of ammonia or an ammonia-producing substance comprising a thinner liquid blocking layer retaining a barrier performance same as that of a conventional thicker liquid blocking layer, resulting in a stable fabrication on the same line used to fabricate other type of analytical elements.

[0008] The purpose of the invention has been accomplished by means of an integral multilayer analytical element for use in the analysis of ammonia or an ammonia-producing substance in liquid samples comprising a transparent support, an indicator layer containing an indicator which produces a detectable change by reaction with gaseous ammonia, a gaseous ammonia-permeable liquid blocking layer, a reagent layer containing an alkaline buffering agent and optionally a reagent capable of reacting with said ammonia-producing substance to produce ammonia, and a spreading layer laminated in this order, characterized in that the liquid blocking layer is composed of at least two porous membranes.

[0009] In the analytical element in accordance with the invention, diameter of pores in the uppermost porous membrane composing the liquid blocking layer, which contacts the reagent layer, is equal to or smaller than that in the second porous membrane from the top. Thus, though the whole thickness of the liquid blocking layer is rather small, liquid blocking properties of the layer can be still maintained. Further, the sensitivity of the analytical element can be also maintained by keeping diameter of pores in the second porous membrane large or by changing material of the second membrane, in spite of small diameter of pores in the uppermost porous membrane.

DESCRIPTION OF THE PREFERED EMBODYMENT

[0010] As a support of the analytical element in accordance with the invention, hydrophobic transparent supports which are generally used in such analytical elements and are made of polymers, such as polyethylene terephthalate, polycarbonate and polyvinyl compounds may be used. Thickness of the support is in a range of abut 50 to 1000 μm, typically about 80 to 300 μm.

[0011] On the support, an indicator layer is provided. The indicator layer contains one or more compounds which change in absorption wavelength as a result of the reaction with gaseous ammonia (hereinafter, the compound is referred to as a dye precursor). The dye precursor which may be used in the analytical element of this invention includes leuco dyes, such as leucocyanine dye, nitro-substituted leuco dye and leucophthalein dye described in U.S. Pat. No. Re. 30,267, pH indicators, such as Bromophenol Blue, Bromo-

cresol Green, Bromothymol Blue, Qinoline Blue and rosolic acid (see "Kagaku Dai-Jiten" (Chemical Dictionary) Kyoritsu, vol. 10, 63-65), triarylmethane dye precursors, leucobenzilidene pigments (see JP 1982-145273 A), diazonium salts and azo dye couplers, base bleachable dyes, and the like.

[0012] At least one dye precursor mentioned above is mixed with water soluble or an organic solvent soluble binder polymer and coated on the transparent support, then dried to provide the indicator layer. Binder polymers for this purpose include polyvinyl alkyl ethers, such as polyvinyl methyl ether, polyvinyl ethyl ether and polyvinyl isobutyl ether, gelatins, such as acid-processed gelatin, alkali-processed gelatin and de-ionized gelatin, cellulose esters, such as cellulose acetate, cellulose acetate butyrate and cellulose acetate propionate, alkyl celluloses, such as methyl cellulose, ethyl cellulose and propyl cellulose, synthetic vinyl polymers, such as polymethylmethacrylate, polyacrylate, polystyrene, polyacrylonitril, polyvinylacetate, polyvinylbutyral, chlorinated polyvinylacetate, polyacrylamide, polyvinylpyrrolidone, polyvinylalcohol and copolymers thereof.

[0013] The dye precursor may be used in a range of about 0.1 to 50%, preferably about 0.5 to 20%, based on the weight of the binder. In order to adjust the sensitivity, various buffering agents, organic or inorganic acids may be used to control the pH. The buffering agent may be selected from those mentioned later. As to organic or inorganic acids, ethanesulfonic, asparaginic, azelaic, glutaric, succinic, glutaconic, tartaric, pimelic, malonic, malic, 3,3-dimethyl glutaric, citric, p-toluenesulfonic, perchloric, hydrochloric acid, and the like may be used. In addition, alkalis, such as sodium hydroxide, potassium hydroxide, sodium carbonate, sodium bicarbonate, and the like may be added to the indicator layer. Organic solvents, such as acetone, 2-methoxy ethanol, methyl ethyl ketone, methanol and ethanol, water or mixtures of them may be preferably used to prepare the coating solution for the indicator layer. The coating solution contains solid ingredients such as the dye precursor, binder polymer etc. in a solid concentration of 1 to 30%, preferably 3 to 20%, by weight to prepare the coating solution. The coating solution is coated on the transparent support and dried to form the indicator layer having a dry thickness in a range of about 1 to 30 μm, preferably about 2 to 20 μm.

[0014] On the indicator layer, a liquid blocking layer is provided. The liquid blocking layer is composed of a microporous substance having pierced voids which are substantially impermeable to liquids, such as a coating solution, a sample solution etc. and interfering ingredients (e.g. alkaline ingredients) dissolved in these liquids and permeable to gaseous ammonia during fabrication and/or analytical operation of the multilayer analytical element.

[0015] The liquid blocking layer in accordance with the present invention is composed of at least two porous membranes, and is characterized in that the diameter of pores in the uppermost porous membrane, which contacts the reagent layer, is equal to or smaller than that in the porous membrane just underlying the uppermost porous membrane. Specifically the diameter of pores in the uppermost membrane is in a range of about 0.01 to 1 μm, preferably 0.04 to 0.2 μm, and the diameter of pores in the membrane just underlying the uppermost membrane is in a range of about 0.2 to 20 μm, preferably about 0.5 to 10 μm. In addition, the former: the latter ratio is in a range of 0.001 to 1.0, preferably 0.01 to 0.5. And now, the diameter of pores in this specification means the average diameter of pores, provided that there is no other particular description.

[0016] There is no particular restriction on material of the porous membrane. Polyethylene, polypropylene, fluorine containing polymers such as polytetrafluoroethylene etc., cellulose acetate, polysulfone, polyamides(nylons), and combinations of them can be mentioned as examples. Among them, combination of polyethylene and polypropylene porous membranes is preferable.

[0017] Each porous membrane has thickness in a range of about 3 to 40 μm, preferably 5 to 20 μm. At least two layers of membrane, generally two to three layers of membrane are combined to form the liquid blocking layer. The liquid blocking layer has a whole void ratio in a range of about 25 to 90%, preferably about 35 to 90%, and a whole thickness in a range of about 10 to 50 μm, preferably about 10 to 30 μm.

[0018] The above mentioned porous membrane is adhered to the aforementioned indicator layer in practical adhesion force. The porous membrane is pasted up to the surface of the indicator layer in wet condition, and dried. Here, wet condition means that the binder of the indicator layer is in a condition of swelling, dispersion or solution by virtue of a residual solvent dissolving the binder or by wetting the dry layer with a dissolving solvent.

[0019] Porous membranes composing the liquid blocking layer may be adhered each other in point contact with a physical and/or chemical technique, such as thermo compression bonding or adhesion using a hot melt adhesive etc. Porous membranes may either be sequentially laminated on the indicator layer or be laminated each other prior to adhesion to the indicator layer.

[0020] On the liquid blocking layer, a reagent layer is provided. The reagent layer is the layer usually containing a reagent reacting with the ammonia-producing substance to produce ammonia (generally an enzyme or a reagent containing an enzyme), an alkaline buffering agent for efficiently releasing the ammonia produced during the reaction in a form of gaseous ammonia, and a hydrophilic polymer binder having a film-forming facility. Examples of combination of ammonia-producing substance/reagent are urea/urease, creatinine/creatinine deiminase, amino acid/amino acid dehydrogenase, amino acid/amino acid oxidase, amino acid/amino acid dehydratase, amino acid/ammonia lyase, amine/amine oxidase, diamine/amine oxidase, glucose and phosphoamidate/phosphoamidate hexose phosphotransferase, ADP/carbamate kinase and carbamoylphosphate, acid amide/amide hydrolase, nucleobase/deaminase, nucleoside/deaminase, nucleotide/deaminase, guanine/guanase, and the like.

[0021] Alkaline buffering agents in the range of pH 7.0 to 10.5, preferably 7.5 to 10.0, are usually usable for the reagent layer. Specific examples of buffering agents are ethylenediaminetetraacetic acid (EDTA), tris(hydroxymethyl)aminomethane (Tris), phosphate buffering agents, N,N-bis(2-hydroxyethyl)glycine (Bicine), Good's buffering agents, such as N-2-hydroxyethylpiperazine-N'-2-hydroxypropane-3-sulfonic acid (Heppso) and N-hydrosyethylpiperazine-N'-ethanesulfonic acid (Hepes) etc., borate buffering agents, and the like.

[0022] Examples of the hydrophilic polymer binder having a film-forming facility usable for the reagent layer include gelatin, agarose, polyvinyl alcohol, polyacrylamide, hydroxymethyl cellulose, hydroxyethyl cellulose, polyvinyl pyrrolidone, and the like.

[0023] The reagent layer may contain, if necessary, a wetting agent, a binder-crosslinking agent (a curing agent), stabilizer, a heavy metal ion-trapping agent (a complexing agent) in addition to the reagent capable of reacting with the ammonia-producing substance to form ammonia, the alkaline buffering agent and the film-forming hydrophilic polymer binder.

[0024] The reagent layer can be formed by preparing a coating solution by mixing the reagent capable of reacting with the ammonia-producing substance to form ammonia, the alkaline buffering agent and optionally other reagents mentioned above with a film-forming hydrophilic binder such as gelatin, applying it on the liquid blocking layer, then drying it.

[0025] The reagent capable of reacting with the ammonia-producing substance to form ammonia is used usually in a range of about 0.1 to 50% by weight, preferably about 0.2 to 20% by weight based on the weight of the binder. The alkaline buffering agent is used appropriately in a range of about 0.1 to 60% by weight based on the weight of the binder. Generally the dry thickness of the reagent layer is in a range of about 1 to 40 μm, preferably about 2 to 20 μm.

[0026] On the reagent layer, a spreading layer is provided. The spreading layer may be a woven fabric spreading layer disclosed in U.S. Pat. Nos. 4,292,272, 4,783,315, etc. (e.g. plain weaves including broad cloth and poplin), a knitted fabric spreading layer disclosed in EP 0 162 302 A, etc. (e.g. tricot, double tricot or milanese), a spreading layer made of organic polymer fiber pulp-containing paper disclosed in JP 1982-148250 A, a fibrous microporous spreading layer, such as spreading layers formed by coating a fluid dispersion of fibers and a hydrophilic polymer disclosed in JP 1982-125847 A etc., a membrane filter (blushed polymer layer) disclosed in U.S. Pat. No. 3,992,158, a continuous microspaces-containing isotropic porous spreading layers where fine particles, such as polymer particulates are joined in point contact with a hydrophilic polymer binder, a non-fibrous isotropic porous spreading layer, such as a continuous microspaces-containing porous spreading layer where polymer particulates are joined in point contact with a polymer adhesive which does not swell in water (three-dimensional lattice structure layer) disclosed in U.S. Pat. No. 4,258,001 etc., a spreading layer with a good blood cell-separatig ability where plural porous layers (for example, two layers including woven or knitted fabric and membrane filter, three layers including woven or knitted fabric, membrane filter, and woven or knitted fabric) are adhered each other to form a laminate using an adhesive laid on their interfaces in discontinuous points or islands (so called halftone dots in printing field) disclosed in U.S. Pat. No. 5,019,347, JP 1987-138756 A, JP 1987-138757 A, EP 0 226 465 A etc.

[0027] Woven fabric or knitted fabric used for the spreading layer can be rendered hydrophilic by processing at least one surface of it with physical activation treatment represented by glow discharge or corona discharge disclosed in U.S. Pat. No. 4,783,315, degreasing by washing with water or impregnating with a hydrophilic polymer disclosed in JP 1980-164356 A, JP 1982-066359 A etc., or by sequential processing of an appropriate combination of these treatments, resulting in a increased adhesion force to the layer located on the underside, i.e. near the support. In addition, a polymer-containing aqueous solution or a polymer-containing mixed solution of water and an organic solvent can be coated on the spreading layer to control expansion area or spread of a liquid sample as disclosed in JP 1984-171864 A, JP 1985-222769 A, JP 1985-222770 A etc.

[0028] Between the reagent layer and the spreading layer, a color-blocking layer or a light-reflective layer may be provided. The color-blocking layer or the light-reflective layer is a layer composed of white particulates, such as titan dioxide particulates or barium sulfate particulates, etc. almost uniformly dispersed in a hydrophilic polymer binder such as gelatin, having light-blocking property or both light-blocking and light-reflecting properties and a dry thickness in a range of about 2 to 20 μm.

[0029] In addition, a known adhesive layer composed of a hydrophilic polymer can be provided on the reagent layer, the color-blocking layer or the light-reflective layer for the purpose of strong adhesion of the spreading layer to form a laminate. The adhesive layer has a dry thickness in the range of about 0.5 μm to5 μm.

[0030] A surfactant may be added to the reagent layer, the color-blocking layer or light-reflecting layer, the adhesive layer, the spreading layer, or the like. A nonionic surfactant may be mentioned as an example. Specific examples of the nonionic surfactant are p-octylphenoxypolyethyoxyethanol, p-nonylphenoxypolyethoxyethanol, polyoxyethylene oleyl ether, polyoxyethylenesorbitanmonolaurate, p-nonylphenoxypolyglycidol, octylglucoside, and the like. By adding the nonionic surfactant to the spreading layer, the spreading action (metering action) for spreading an aqueous liquid sample is improved. By adding the nonionic surfactant to the reagent layer, the color-blocking layer or light-reflecting layer, or the adhesive layer the water in an aqueous liquid sample is easily and substantially uniformly absorbed by the reagent layer during analytical operations, and the liquid contact with the spreading layer becomes rapid and substantially uniform.

[0031] The analysis of ammonia or ammonia-producing substance in a liquid sample using the integral multilayer analytical element in accordance with the invention can be conducted according to a following analytical operation sequence; spot a liquid sample, such as whole blood, plasma, serum, urine, etc. on the spreading layer in a range of 3 to 30 μL, preferably 6 to 15 μL; incubate the spotted element at a substantially constant temperature in a range of about 20° C. to 40° C. for a period in a range of 1 to 10 minutes; measure the degree of color change (coloring or discoloration) occurred in the indicator layer by reflection photometry through the transparent support, or visually compare the hue in the indicator layer with a standard hue.

EXAMPLES

[0032] Example 1

[0033] The following indicator layer was applied in a form of an ethanol solution onto a transparent polyethylene terephthalate (PET) film having a thickness of 180 μm, and dried.

Indicator Layer	
Bromophenol Blue	110 mg/m²
Polyvinyl ethyl ether	1.8 g/m²
(Weight average molecular weight: about 40,000)	
Sodium hydroxide	7 mg/m²

[0034] On the indicator layer, the porous membrane ① shown in the Table 1 was uniformly pressed to provide a liquid-blocking layer. On the liquid-blocking layer, a following reagent layer was applied in a form of a aqueous solution, and dried. On this occasion, the layer 1 of the porous membrane ① was arranged so as to contact the reagent layer.

Reagent Layer	
Hydroxyethyl cellulose	14 g/m²
(Mean molecular weight: about 40,000)	
mean substitution degree of hydroxyethyl group: DS = 1.0 to 1.3	
mean number of moles: MS = 1.8 to 2.5	
Sodium tetraborate	4 g/m²
(pH of the coating solution: 10.0)	

[0035] Immediately after the above reagent layer was almost uniformly wetted with 0.2% p-nonylphenoxypolyglycidol aqueous solution, a knitted polyester fabric (gauge number: 40) was pressed uniformly to form a laminate.

[0036] In addition, polyvinylpyrrolidon was impregnated into the laminate by applying the following ethanol solution for the purpose of improving spreading property, and dried to complete an integral multilayer analytical element for the determination of ammonia.

Polyvinylpyrrolidone	6.8 g/m²
(mean molecular weight: about 1,200,000)	

[0037] Example 2

[0038] An integral multilayer analytical element for the determination of ammonia was prepared similar to Example 1, except that the membrane ② shown in the Table 1 was used in place of membrane ①.

[0039] Comparative Example 1

[0040] An integral multilayer analytical element for the determination of ammonia was prepared similar to Example 1, except that the membrane ③ shown in the Table 1 was used in place of membrane ①.

[0041] Comparative Example 2

[0042] An integral multilayer analytical element for the determination of ammonia was prepared similar to Example 1, except that the membrane ④ shown in the Table 1 was used in place of membrane ①.

TABLE 1

	Construction of Porous Membranes ① to ④			
	Membrane①	Membrane②	Membrane③	Membrane④
Layer 1				
thickness (μm)	9	9	25	25
pore diameter (μm)	0.1	0.1	0.1	1 to 3
material	polypropylene	polypropylene	polyethylene	polyethylene
Layer 2				
thickness (μm)	7	7		
pore diameter (μm)	1 to 3	1 to 3	—	—
material	polyethylene	polyethylene		
Layer 3				
thickness (μm)		9		
pore diameter (μm)	—	0.1	—	—
material		polypropylene		
Mean Void Ratio	44	40	32	65

[0043] Evaluation of Integral Multilayr Analytical Elements for TEH Determination of Ammonia

[0044] Integral multilayer analytical elements for the determination of ammonia of Examples 1 and 2 or Comparative Examples 3 and 4 above mentioned were evaluated by the following method.

[0045] Aqueous ammonium sulfate solutions were prepared so as to contain ammonia nitrogen in concentration of 0, 60, 200 or 400 μg/dL, respectively, to provide solutions for evaluation test.

[0046] On the spreading layer of respective analytical elements, 10 μL of each solution for evaluation test was spotted. After two minutes, optical density (OD) of developed color was measured at 600 nm by the reflection photometry. Then a calibration curve was prepared by plotting the measured optical density versus the ammonia nitrogen concentration. Besides, the above coloring test was

repeated ten times as to each analytical element and each solution for evaluation test, and respective optical density was measured. Each measured optical density was converted to ammonia nitrogen concentration by using the above calibration curve, and each coefficient for variation (CV) of the converted values was determined. Results are represented in the Table 2.

TABLE 2

		Ammonia Nitrogen Concentration (μ g/dL)			
		0	50	200	400
Example 1	OD:	0.39	0.48	0.72	0.99
	CV (%)	—	2.2	2.0	1.3
Example 2	OD:	0.34	0.41	0.63	0.88
	CV (%):	—	1.74	1.24	2.26
Comparative Example 1	OD:	0.34	0.38	0.55	0.76
	CV (%)	—	8.1	3.5	5.2
Comparative Example 2	OD:	0.44	0.98	1.25	1.40
	CV (%):	—	90.3	43.2	41.7

[0047] As shown in Table 2, the sensitivity of the analytical elements of Examples 1 and 2 is higher than that of Comparative Examples 1 and 2, and moreover, the measuring accuracy of them is also improved. Further, the liquid blocking layer of Comparative Example 2 does not work as is expected, and so the solution for evaluation test spotted to the reagent layer reached the indicator layer, resulting in significant deterioration of CV.

[0048] According to this invention, a compacter slide can be obtained by using a thinner porous membrane, and so it is stably fabricated since the whole thickness of it corresponds with those of other slides.

1. In an integral multilayer analytical element for the determination of ammonia or an ammonia-producing substance comprising a transparent support, an indicator layer containing an indicator which produces a detectable change by gaseous ammonia, a liquid blocking layer permitting a gaseous ammonia to pass therethrough, a reagent layer containing an alkaline buffering agent and optionally a reagent capable of reacting with said ammonia-producing substance to produce ammonia, and a spreading layer, adhesively laminated in this order, the improvement which comprises that said liquid blocking layer is composed of at least two porous membrane layers.

2. The integral multilayer analytical element as claimed in claim 1, wherein pore diameter in the uppermost porous membrane of said at least two porous membrane layers, which contacts said reagent layer, is equal to or smaller than that of a just underlying porous membrane.

3. The integral multilayer analytical element as claimed in claim 1, wherein said at least two porous membrane layers comprise a porous polypropylene membrane and a porous polyethylene membrane.

* * * * *

DOCUMENT-IDENTIFIER: US 5174959 A
**** See image for Certificate of Correction ****
TITLE: Breath component monitoring device

Abstract Text (1):
The present invention relates to methods and materials for the detection of ketone and aldehyde analytes in fluid samples by means of reacting analyte containing samples with a first solid matrix material to which a nitroprusside salt is coupled and a second solid matrix material to which an amine is covalently coupled. Methods and devices are also provided for ascertaining the fat catabolism effects of a weight loss dietary regimen comprising determining the breath acetone concentration of the subject.

Brief Summary Text (9):
Many assays take advantage of the "Legal" method which utilizes the reaction of a carbonyl group containing compound such as a ketone or an aldehyde with a nitroprusside (nitroferricyanide) salt in the presence of an amine to form a colored complex. While acetone will react, albeit slowly, with nitroprusside under aqueous conditions, the reaction of acetoacetic acid is some 100 to 200 times faster with the result that "Legal" reactions under aqueous conditions whether detecting "acetone," "acetone bodies" or "ketone bodies" primarily detect acetoacetic acid. The color reaction is believed to occur as a result of a coupling reaction through the nitroso group of the nitroprusside with the analyte to form an intermediate which then complexes with the amine to produce a color characteristic of the specific amine. In forming the complex, the trivalent iron of the nitroprusside is reduced to its divalent state. The color complex, however, is unstable because nitroprusside decomposes rapidly in alkaline solutions. Further, nitroprusside salts are subject to decomposition in the presence of moisture and high pH. Frequently during storage, a brown decomposition product is formed which can interfere with sensitive detection during assays. These limitations have led to numerous attempts to stabilize the color complex by utilizing mixtures of nitroprussides and amines or amino acids in combination with a variety of buffers, metal salts, organic salts, organic stabilizers and polymers. Numerous combinations of reagents have been shown to be suitable for detection of a variety of ketone bodies in liquid samples although the analyte predominantly detected in physiological fluids is acetoacetic acid.

Brief Summary Text (10):
Fortune, U.S. Pat. No. 2,186,902 discloses the use of soluble nitroprusside chromogens in the presence of ammonia and soluble carbonates for the detection of what was termed "acetone" (actually acetoacetic acid) in urine samples. Varying colorations are observable for the quantitative determination of "acetone" levels.

Detailed Description Text (2):
The present invention comprises methods and materials for the determination of fluid ketone and aldehyde concentrations through the reaction of such carbonyl group containing compounds with a nitroprusside compound in the presence of an amine and a suitable solvent to produce a color reaction Devices according to the invention comprise a first solid matrix material to which a nitroprusside salt is coupled and a second solid matrix material to which an amine is covalently bound. The addition of magnesium or calcium salts in the test composition promotes chelate formation thus stabilizing the color product and enhancing the kinetics of the reaction between the carbonyl compound, the amine and the nitroprusside.

Detailed Description Text (13):
Amines suitable for covalent binding to the second solid matrix materials of the present invention include those amines capable of reacting with ketones or aldehydes and nitroprusside materials in the presence of a solvent to produce a detectable color complex Suitable amines include primary and

secondary polyamines and primary and secondary lower alkyl amines with from 1 to 10 carbons. Primary amines are preferred although secondary amines are also suitable for methods and procedures of the present invention Amines are coupled to the second solid matrix materials of the invention by means of coupling moieties Typically the matrix materials are reacted with silane substituted amine-coupling agent conjugates such as 3'-aminopropyltrimethoxysilane. This material will react with a suitable matrix material such as silica gel or cellulose to produce aminopropyl silica gel or aminopropyl cellulose although the invention is not limited to aminopropyl moieties and other materials are equally suitable.

Detailed Description Text (97):
Concentrations of ketones and aldehydes present in liquid samples may be determined utilizing the same methods and materials of the invention used for analysis of vapor. According to well known procedures, however, head space vapor in equilibrium with the liquid sample to be analyzed is collected and analyzed according to procedures for analyzing vapor samples Ketone and aldehyde vapor concentrations may be related to liquid sample concentrations through use of known vapor pressure and partition coefficient relationships. Head space analysis is useful for the determination of concentrations of more volatile ketone and aldehyde sample components and is particularly useful for the determination of acetone concentrations in aqueous samples. Detection of acetone in such aqueous samples is otherwise hampered by the interference of water with the nitroprusside/amine color reaction. Vapor collected by head-space analysis may be desiccated according to the methods disclosed above in order to prevent the adverse effects of water on the color reaction.

CLAIMS:

12. A kit for the determination of levels of various components in a user's breath, said kit comprising:

a) a portable housing with an opening at one end and containing:

i) an outer tubular member having an open first end and a second end, wherein said outer tubular member is contained within said portable housing such that said open first end of said outer tubular member is in communication with said portable housing opening;

ii) valve means disposed toward said second end of said outer tubular member; and

iii) a breath sample collection means having one end mounted on said outer tubular member and having the other end closed, and having an interior in communication with the inside of said outer tubular member through said valve means for collecting a fixed volume of a user's breath; and

b) a disposable tubular analyzer column containing material reactive to the presence of at least one breath component, wherein said column is insertable into said outer tubular member through said portable housing opening, co-operates with said valve means to permit breath blown by a user into said outer tubular member to pass through said outer tubular member into said breath sample collection means and to permit said collected breath sample to discharge through the reactive material of said analyzer column.

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